SEP 22 1923
Medical Library

THE JOURNAL
OF
GENERAL PHYSIOLOGY

EDITORS
JACQUES LOEB W. J. V. OSTERHOUT

VOLUME VI, No. 1. SEPTEMBER 20, 1923

PUBLISHED BIMONTHLY
AT MOUNT ROYAL AND GUILFORD AVENUES, BALTIMORE, MD.
BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

COPYRIGHT, 1923, BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

Entered as second-class matter November 25, 1918, at the Post Office at Baltimore, Md., under the Act of March 3, 1879.

Made in the United States of America

PUBLICATIONS OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

THE JOURNAL OF EXPERIMENTAL MEDICINE

EDITED BY

SIMON FLEXNER, M.D. PEYTON ROUS, M.D.

The Journal of Experimental Medicine is designed to cover the field of experimental medicine. It is issued monthly, two volumes of over 600 pages each appearing in a year. The subscription price per year (two volumes) is $5.00, payable in advance. Single copies cost 75 cents. Remittances should be made by draft or check on New York, or by postal money order, payable to *The Journal of Experimental Medicine*, Mount Royal and Guilford Avenues, Baltimore, Md., or Avenue A and 66th Street, New York, N. Y.

THE JOURNAL OF BIOLOGICAL CHEMISTRY

EDITED BY

STANLEY R. BENEDICT, New York, N. Y. LAFAYETTE B. MENDEL, New Haven, Conn.
H. D. DAKIN, Scarborough, N. Y. DONALD D. VAN SLYKE, New York, N. Y.

The Journal of Biological Chemistry is designed for the prompt publication of original investigations of a chemical nature in the biological sciences. It is issued monthly. Each volume consists of as many numbers as are required to make a total of approximately 800 pages. The subscription price is $5.00 per volume to subscribers in the United States and Canada, payable in advance; $5.25 to those in other countries. The number of volumes issued yearly averages four. The price of single copies varies; it is given on the back cover of each number. Remittances should be made by draft or check on New York, or by postal money order, payable to *The Journal of Biological Chemistry*, Mount Royal and Guilford Avenues, Baltimore, Md., or Avenue A and 66th Street, New York, N. Y.

THE JOURNAL OF GENERAL PHYSIOLOGY

EDITED BY

JACQUES LOEB, New York, N. Y. W. J. V. OSTERHOUT, Cambridge, Mass.

The Journal of General Physiology is devoted to the explanation of life phenomena on the basis of the physical and chemical constitution of living matter. Information regarding contributions and subscriptions is given in full on the back cover.

STUDIES FROM THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

The published results of investigations conducted in the Institute, or under its grants, are republished at irregular intervals in volumes designated *Studies from The Rockefeller Institute for Medical Research*. The subscription price is $2.00 per volume, payable in advance. A special rate of $1.00 per volume is made to those who subscribe to *The Journal of Experimental Medicine*, *The Journal of Biological Chemistry*, or *The Journal of General Physiology*. Remittances should be made by draft or check on New York, or by postal money order, payable to The Rockefeller Institute for Medical Research, Avenue A and 66th Street, New York, N. Y.

SEMIANNUAL LIST

The Institute publishes a *Semiannual List* of all papers by members of the staff and those working under grants from the Institute, stating the title and place of publication of these reports. The *Semiannual List* will be sent regularly upon application.

CONTENTS.

No. 1, SEPTEMBER 20, 1923.

PAGE

HUSSEY, RAYMOND G., and THOMPSON, WILLIAM R. The effect of radioactive radiations and x-rays on enzymes. II. The effect of radiations from radium emanation on pepsin in solution..... 1

HUSSEY, RAYMOND G., and THOMPSON, WILLIAM R. The effect of radioactive radiations and x-rays on enzymes. III. A unit of measure of activity for radium emanation..... 7

HARVEY, E. NEWTON, and MORRISON, THOMAS F. The minimum concentration of oxygen for luminescence by luminous bacteria..... 13

BRODY, SAMUEL, RAGSDALE, ARTHUR C., and TURNER, CHARLES W. The rate of growth of the dairy cow. III. The relation between growth in weight and increase of milk secretion with age..... 21

BRODY, SAMUEL, RAGSDALE, ARTHUR C., and TURNER, CHARLES W. The rate of growth of the dairy cow. IV. Growth and senescence as measured by the rise and fall of milk secretion with age..... 31

BRODY, SAMUEL, HENDERSON, EARL W., and KEMPSTER, H. L. The rate of senescence of the domestic fowl as measured by the decline in egg production with age..... 41

HOAGLAND, D. R., and DAVIS, A. R. Further experiments on the absorption of ions by plants, including observations on the effect of light..... 47

EGGERTH, ARNOLD H. Changes in the stability and potential of cell suspensions. I. The stability and potential of *Bacterium coli*..... 63

MOORE, A. R. The function of the brain in locomotion of the polyclad worm, *Yungia aurantiaca*..... 73

FULMER, ELLIS I., and BUCHANAN, R. E. Studies on toxicity... 77

HILL, A. V. Membrane potentials and colloidal behavior..... 91

HITCHCOCK, DAVID I. Membrane potentials and colloidal behavior. Reply to the note by Professor A. V. HILL..... 93
HITCHCOCK, DAVID I. The combination of deaminized gelatin with hydrochloric acid..... 95
LOEB, JACQUES. On the location of the forces which determine the electrical double layer between collodion particles and water..... 105

No. 2, NOVEMBER 20, 1923.

LANDSTEINER, KARL, and HEIDELBERGER, MICHAEL. Differentiation of oxyhemoglobins by means of mutual solubility tests..... 131
HARTLINE, H. KEFFER. Influence of light of very low intensity on phototropic reactions of animals..... 137
PARSONS, THOMAS R., and PARSONS, WINIFRED. Observations on the transport of carbon dioxide in the blood of some marine invertebrates..... 153
PLOUGH, HAROLD H., and STRAUSS, MAURICE B. Experiments on toleration of temperature by *Drosophila*..... 167
WINSLOW, C.-E. A., FALK, I. S., and CAULFIELD, M. F. Electrophoresis of bacteria as influenced by hydrogen ion concentration and the presence of sodium and calcium salts.... 177
HITCHCOCK, DAVID I. Conductivity titration of gelatin solutions with acids..... 201
LOEB, JACQUES. Theory of regeneration based on mass action. II..... 207
LOEB, JACQUES. The influence of the chemical nature of solid particles on their cataphoretic P.D. in aqueous solutions..... 215

No. 3, JANUARY 20, 1924.

NORTHROP, JOHN H. The kinetics of trypsin digestion. I. Experimental evidence concerning the existence of an intermediate compound..... 239
BRODY, SAMUEL. The kinetics of senescence..... 245
ROBBINS, WILLIAM J. Isoelectric points for the mycelium of fungi..... 259
HEYMANS, C., and MOORE, A. R. Luminescence in *Pelagia noctiluca*..... 273

MOORE, A. R. The nervous mechanism of coordination in the crinoid, *Antedon rosaceus* 281
CROZIER, W. J. Hydrogen ion concentrations within the alimentary tract of insects 289
COLE, WILLIAM H. The effect of laboratory age upon the phototropic reactions of *Limulus* 295
LYON, CHARLES J. The effect of phosphates on respiration 299
LOEB, JACQUES. Hydrophilic and hydrophobic colloids and the influence of electrolytes on membrane potentials and cataphoretic potentials 307
BRODY, SAMUEL, and RAGSDALE, ARTHUR C. The rate of growth of the dairy cow. V. Extrauterine growth in linear dimensions 329
NORTHROP, JOHN H. A test for diffusible ions. I. The ionic nature of trypsin 337

No. 4, MARCH 20, 1924.

LASAREFF, P. Ionic theory of activity of nerve centers and of propagation of nerve impulse 349
HECHT, SELIG. Intensity discrimination and the stationary state 355
FRICKE, HUGO. A mathematical treatment of the electrical conductivity of colloids and cell suspensions 375
MOORE, MARY M. Tropistic reactions of *Cerianthus membranaceus* 385
MOORE, MARY M. The reaction of *Cerianthus* to two sources of light 393
MOORE, A. R. Luminescence in *Mnemiopsis* 403
KUNITZ, M. A cell for the measurement of cataphoresis of ultramicroscopic particles 413
NORTHROP, JOHN H. The kinetics of trypsin digestion. II. Conditions under which the reaction is monomolecular 417
NORTHROP, JOHN H. The kinetics of trypsin digestion. III. The course of the reaction with constant substrate under conditions causing inactivation of the enzyme 429
NORTHROP, JOHN H. The kinetics of trypsin digestion. IV. The course of the reaction when both substrate and enzyme concentrations are decreasing 439

LOEB, ROBERT F. On the diffusibility of the calcium of blood serum through collodion membranes. The effect of sodium chloride and changes in hydrogen ion concentration..... 453
HITCHCOCK, DAVID I. The isoelectric point of gelatin at 40°C.. 457
LOEB, JACQUES. Theory of regeneration based on mass relation. III. Further experiments on the cause of the polar character of regeneration..... 463
LOEB, JACQUES, and KUNITZ, M. The ultimate units in protein solutions and the changes which accompany the process of solution of proteins..... 479

No. 5, MAY 20, 1924.

MCCUTCHEON, MORTON, and LUCKE, BALDWIN. The mechanism of vital staining with basic dyes..... 501
RAY, GEORGE B. The oxidation of sodium lactate by hydrogen peroxide..... 509
RAY, GEORGE B. The effect of cystine and glycocoll on the oxidation of sodium lactate by hydrogen peroxide..... 525
CROZIER, W. J. On stereotropism in *Tenebrio* larvæ..... 531
BRODY, SAMUEL, TURNER, CHARLES W., and RAGSDALE, ARTHUR C. The relation between the initial rise and the subsequent decline of milk secretion following parturition 541
KUNITZ, M. Valency rule and alleged Hofmeister series in the colloidal behavior of proteins. III. The influence of salts on osmotic pressure, membrane potentials, and swelling of sodium gelatinate..... 547
CHAPMAN, ROYAL N. Nutritional studies on the confused flour beetle, *Tribolium confusum* Duval..... 565
EGGERTH, ARNOLD H. Changes in the stability and potential of cell suspensions. II. The potential of erythrocytes..... 587
UHLENHUTH, EDUARD. The growth of the thyroid and postbranchial body of the salamander, *Ambystoma opacum*..... 597
NORTHROP, JOHN H., and FREUND, JULES. The agglutination of red blood cells..... 603
WIBLE, CHARLES L. The locus of the action of veratrin..... 615

No. 6, JULY 20, 1924.

DU NOÜY, P. LECOMTE. Surface tension of serum. XI. An improvement of the technique for measuring surface tension. Plate 1..... 625

BRODY, SAMUEL. A note on the similarities between the curves of growth and of regeneration..... 629

GRANT, J. H. B., and GATES, FREDERICK L. The effect on the external parathyroid glands of the exposure of rabbits to ultra-violet light. Plate 2..... 635

CROZIER, W. J. Wave length of light and photic inhibition of stereotropism in *Tenebrio* larvæ..... 647

EYSTER, W. H., and ELLIS, M. M. Growth of maize seedlings as affected by glucokinin and insulin..... 653

MACHT, DAVID I., and HILL, EBEN C. The effect of ultra-violet, x-ray, and radium radiation on the toxicity of normal blood..... 671

BRINK, R. A. The influence of electrolytes on pollen tube development..... 677

GRIFFITH, FRED R., JR. The effect of potassium on the acid metabolism of surviving skeletal and cardiac muscles of the frog..... 683

WINSLOW, C.-E. A., and SHAUGHNESSY, H. J. The alkaline isopotential point of the bacterial cell..... 697

CROZIER, W. J., and MANGELSDORF, A. F. A note on the relative photosensory effect of polarized light..... 703

CROZIER, W. J., and PILZ, G. F. The locomotion of *Limax*. I. Temperature coefficient of pedal activity..... 711

NORTHROP, JOHN H. The kinetics of trypsin digestion. V. Schütz's rule..... 723

HECHT, SELIG. Photochemistry of visual purple. III. The relation between the intensity of light and the rate of bleaching of visual purple..... 731

FRICKE, HUGO. The electric conductivity of disperse systems..... 741

HITCHCOCK, DAVID I. The solubility of tyrosine in acid and in alkali..... 747

MURRAY, CECIL D. Application of the diffusion hypothesis to membrane potentials..... 759

INDEX TO VOLUME VI..... 769

THE EFFECT OF RADIOACTIVE RADIATIONS AND X-RAYS ON ENZYMES.

II. The Effect of Radiations from Radium Emanation on Pepsin in Solution.

By RAYMOND G. HUSSEY and WILLIAM R. THOMPSON.

(From the Laboratory of Biophysical Research of The Memorial Hospital and the Department of Pathology, Cornell University Medical College, New York.)

(Received for publication, June 12, 1923.)

In a previous communication[1] we have reported observations on some aspects of a radiochemical reaction, effected by the radiations (beta and gamma) from radium emanation and its radioactive products in equilibrium with it, in which the enzyme trypsin is inactivated. Our experiments brought out the significant fact that *under definite conditions of irradiation*, the change in the logarithm of the concentration of active trypsin is a linear function of a variable, W, which is algebraically equal to the product of the average amount of radium emanation present, E_a, and the time of exposure, t; *i.e.*, $E_a t = W$.[2] The experimental results obtained are formulated in the following equation.

$$\log_e Q - \log_e Q_o = - kW \qquad (1)$$

Where Q_o is the initial concentration of active trypsin and Q is the concentration of active trypsin found after the solution has been

[1] Hussey, R. G., and Thompson, W. R., *J. Gen. Physiol.*, 1922–23, v, 647.

[2] As explained in a communication immediately following this one, W represents the amount of energy liberated by the radioactive source during a period of exposure and should therefore be measured in energy units. In order to give clear definition to the variables involved in the experiments we have introduced a unit of measure of activity P which we have named the *curie-power*. The amount of emanation in equilibrium and the activity of the preparation are numerically equal, therefore the relation $E_a t = P_a t \equiv W$ exists, and W is measured in terms of the energy unit *curie-power hour*.

irradiated for a given value of W. The concentration of the enzyme is expressed in arbitrary units. When this equation is solved for k we obtain

$$k = \frac{1}{W} \log_e \frac{Q_o}{Q} \tag{2}$$

We have found that pepsin in solution is also inactivated by the radiations discussed. The results of our experiments indicate that the law expressing the quantitative relation between the variables, Q and W, when trypsin solutions are irradiated holds satisfactorily for pepsin solutions too. The data obtained from observations made following the exposures of 4.6 cc. of a 1 per cent solution of pepsin (Fairchild's) to the radiations (beta and gamma) from radium emanation for the "millicurie hour periods" indicated were as follows:

Millicurie hours W	Units of active pepsin found. Q	$k = \frac{1}{W} \log_e \frac{Q_o}{Q}$ $k \times 10^5$
	$Q_o = 1.30$	
2662	1.12	5.61
5900	0.950	5.35
9810	0.776	5.25

The value for Q_o is the average of six observations and the values given for Q are the average of two.

The graphic presentation of the results is given in Fig. 1, where it will be observed that log Q is a linear function of the variable, W, as is required to satisfy equation (1).

Technical Procedure.

The pepsin solution was prepared as follows: 5 gm. of Fairchild's powdered pepsin were scattered over the surface of 100 cc. of 0.06 M hydrochloric acid contained in a 500 cc. pyrex glass beaker. After standing overnight at room temperature, the solution was stirred and poured into a pyrex glass flask. This flask with the pepsin solution was then placed in a water bath for 24 hours. The temperature of the bath was maintained at 34° ± 0.02°C. One part of the resulting solution mixed with four parts of distilled water was the

dilution used in the test. This solution was of about pH 4.2, as determined colorimetrically, and 0.5 cc. contained 1.30 units of active pepsin.

The activity of the pepsin in solution was determined by Northrop's viscosity method.[3] The gelatin solution employed was prepared by adding 25 cc. of M/30 H_3PO_4 to 975 cc. of an aqueous solution of isoelectric gelatin containing a sufficient amount of gelatin to make the final preparation 3 per cent gelatin by dry weight. This solution had a pH of about 4.4, and about 185 seconds were required for 5 cc. to pass through the viscosity pipette.

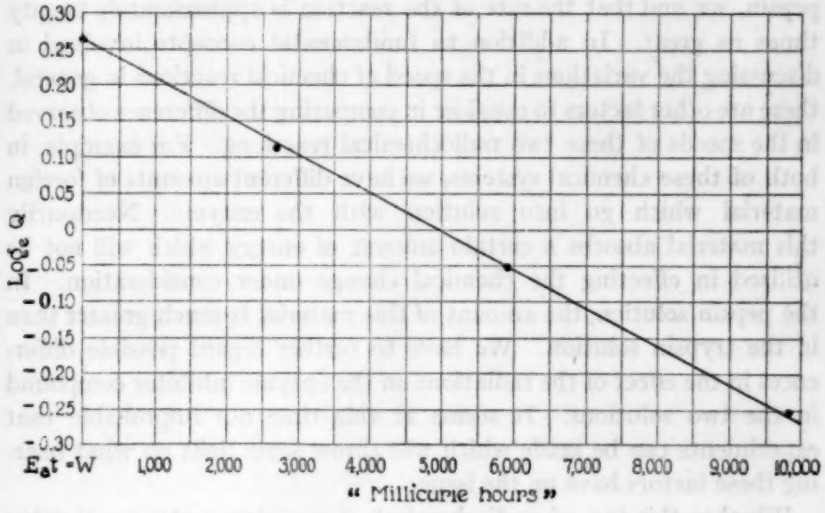

FIG. 1.

The arrangement for irradiating the pepsin solutions differed somewhat from that stated for our previous investigation with trypsin. The radium emanation was contained in a spherical glass bulb which was fused to a length of fine glass rod. The outside diameter of the bulb was approximately 3 mm. The wall was sufficiently thick to prevent the passage of α radiations, but interfered only slightly with the passage of β and γ radiations. In all the tests the pepsin solution was put into a spherical bulb of 4.6 cc. volume and

[3] Northrop, J. H., and Hussey, R. G., *J. Gen. Physiol.*, 1922–23, v, 353.

the small bulb containing the emanation was placed as near the center of the solution as possible. The thickness of the absorbing layer for the radiations emitted from the radium emanation was approximately 9.6 mm. This arrangement permitted the absorption of practically all the β radiations emitted. The bulb containing the test was kept in melting ice during each exposure.

DISCUSSION.

It is of interest to note that when the same volume of dilute trypsin solutions is irradiated under the conditions described above for pepsin, we find that the rate of the reaction is approximately twenty times as great. In addition to fundamental concepts involved in discussing the variations in the speed of chemical reactions in general, there are other factors to consider in comparing the difference observed in the speeds of these two radiochemical reactions. For example, in both of these chemical systems, we have different amounts of foreign material which go into solution with the enzyme. Necessarily this material absorbs a certain amount of energy which will not be utilized in effecting the chemical change under consideration. In the pepsin solution, the amount of this material is much greater than in the trypsin solution. We have to further regard possible differences in the effect of the radiations on the enzyme inhibitor compound in the two solutions. It seems at this time not improbable that experiments can be made which will throw some light on what bearing these factors have on the issue.

Whether this law of radiochemical change has wider application remains to be determined by similar experiments with other chemical systems. If this is found to be the case it would appear that we have a possible method which will permit a quantitative determination of the relative effect of various forms of radiant energy. Further investigations of these radiochemical reactions may lead to the development of a basis for the interpretation of the reactions effected in living matter by these forms of radiant energy.

CONCLUSION.

Pepsin in solution is inactivated by the radiations (beta and gamma) from radium emanation. This chemical effect has been studied quantitatively.

The principles involved in this radiochemical reaction are apparently the same as those found in the case of trypsin previously reported; namely, the change in the logarithm of the concentration of active enzyme varies directly with the variable, W.

THE EFFECT OF RADIOACTIVE RADIATIONS AND X-RAYS ON ENZYMES.

III. A Unit of Measure of Activity for Radium Emanation.

By RAYMOND G. HUSSEY and WILLIAM R. THOMPSON.

(From the Laboratory of Biophysical Research of The Memorial Hospital and the Department of Pathology, Cornell University Medical College, New York.)

(Received for publication, July 30, 1923.)

In our studies on the radiochemical reaction effected by the radiations (beta and gamma) from radium emanation in which enzymes are inactivated we have stated that in a given system the chemical change is a function of the product of two variables; namely, the average amount of emanation present, E_a, and time, t. The product of these two variables has been equated to a single variable, W, for which we have employed the expression "millicurie hours" as a unit of measure. This is not, however, a suitable unit of measure of the quantity of energy which W represents. That no other expression has been available is due to the circumstance that up to the present time but little attention has been given to making clear a distinction between activity and amount of radium emanation. The curie is defined as the amount of emanation in equilibrium with 1 gm. of radium element. In our experiments we are concerned not only with the amount of emanation but more particularly with the *activity* or *power*[1] of the preparation, for which no unit has yet been assigned. In this communication we shall endeavor to show the desirability of having such a unit and suggest what appears to us to be one that will meet our

[1] We use *activity* and *power* synonomously in the sense in which the words are defined in physics. *Cf.* Duff, A. W., A text-book of physics, Philadelphia, 1916, 4th edition, 44. It is necessary that we emphasize this definition since activity is sometimes used in a descriptive sense in discussions on radioactive radiations. Thus, activity is used to denote the "intensity" of electrical, or other effect, of the radiations from one radioactive substance compared to another.

requirements. A simple means of developing a concept for this unit is suggested by the experimental fact that the *activity* of any given preparation of radium emanation is proportional to the amount of emanation present. Indeed, the measurement of *amount* of radium emanation is based on this fact.

Energy is liberated from radium emanation and its radioactive products in equilibrium with it as kinetic energy; contributed by four principal components; namely, alpha, beta, and gamma radiations and recoil atoms. The *rate* of liberation of this energy has been measured by Rutherford by means of converting the kinetic energy of these components into heat. From observations made in an experimental arrangement which permitted nearly complete absorption of the radiations emitted from 1 curie of radium emanation and its radioactive products in equilibrium with it, Rutherford[2] found the rate of liberation of energy expressed in heat units, to be approximately 109.3 gram calories per hour. *This rate of liberation of energy by the above mentioned source we propose to define as a unit of power or activity of such a radioactive source.* As a name for this unit we suggest the expression *curie-power*. Accordingly the *power* or *activity*, which we shall denote as P, of a given preparation of radium emanation, expressed in terms of the unit *curie-power*,[3] is numerically equal to the amount of emanation present, expressed in curies; *i.e.*, the variables, E and P, are algebraically equal. We can express the relation of the variables considered as follows:

$$E_a t = P_a t \equiv W \qquad (1)$$

in which E_a is expressed in units of mass, curies; P_a is expressed in units of *activity*, the *curie-power; t* is time expressed in hours in each case. Then W, representing a quantity of energy, is identical with the product $P_a t$, *i.e.* power and time, is expressed in energy units

[2] Rutherford, E., Radioactive substances and their radiations, Cambridge, 1913, 580.

[3] The unit, *curie-power*, will have the same significance in radium emanation measurements that the unit, candle-power, has in illumination measurements. The appropriateness of this name may be questioned but at the present time it appears to us the most fitting one we can suggest. For practical purposes the *millicurie-power* is used for convenience.

as it should be. Since the *unit of activity* is the *curie-power*, the *energy unit* will be designated the *curie-power hour* and 1 *curie-power hour* is equal to 109.3 gram calories.

We shall now consider the principles involved in the determination of the quantity of radium emanation and indicate that the determination is based on the measure of *relative activity* or *power*. For example, amounts of emanation are determined by comparing the ionizing effect of the gamma radiations emitted by a source of unknown value with the effect of the same kind of radiations emitted by a standard source containing emanation in equilibrium with a known amount of radium element. The ionization is effected in air contained in an ionization chamber and the measurements are made under fixed conditions of observation. When ionization of a gas is effected by these radiations in an electric field where the potential gradient is sufficiently great to practically prevent the recombination of ions produced, the electric current which flows is called the saturation current. The magnitude of this current depends upon the number of pairs of ions produced per unit time and its value is the measure of the ionizing effect of the radiations. It is therefore a measure of the *rate of production of ions*. Since the production of ions depends upon the *ability* of the radiations *to do work* it follows that the number of pairs of ions produced per unit time depends upon *the amount of work done per unit time* by the radiations. Clearly then, in a given system, the rate of production of ions varies with the *power* or *activity* of the radioactive source. When the value for the *relative activity* of two preparations is desired, as in the case of radium emanation measurements, it is necessary that the ionization be effected by radiations of the same kind or quality.

The use of the units suggested above permits us to make a more comprehensive statement of our experimental results already published.[4,5] The relation which was found to exist between the chemical change observed and the variable, W, is stated by the equation

$$\log Q - \log Q_o = -kW \qquad (2)$$

[4] Hussey, R. G., and Thompson, W. R., *J. Gen. Physiol.*, 1922–23, v, 647.

[5] Hussey, R. G., and Thompson, W. R., *J. Gen. Physiol.*, 1923–24, vi, 1.

where the logarithms are to the base e; Q_o is the initial concentration of active enzyme expressed in arbitrary units; Q is the concentration of active enzyme found following an exposure of the enzyme solution to the radiations discussed, for a given increment of energy, W.[6] W is expressed in *millicurie-power hours*. From equation (1) we can write

$$W = P_a t = E_a t$$

Now

$$E_a t = \int_o^t E dt = E_o \int_o^t e^{-\lambda} dt$$

Since

$$E = P, P_o = E_o$$

Whence

$$W = \int_o^t P dt = P_o \int_o^t e^{-\lambda} dt = P_a t \tag{3}$$

In any experiment Q_o is constant, therefore equation (2) may be written

$$\log Q = -kW + C$$

which on differentiation becomes

$$\frac{1}{Q}\frac{dW}{dQ} = -k, \quad \text{or} \quad dQ = -k\,Q\,dW \tag{4}$$

from equation (3) it is evident that $Pdt = dW$ and if we substitute this value of dW in equation (4) we can write the differential equation for the entire experiment; *i.e.*,

$$dQ = -k\,Q\,Pdt \tag{5}$$

---

[6] It is evident from the preceding discussion that W represents the total energy liberated by the radioactive source during the period of exposure. According to Rutherford's data referred to, the energy contributed per hour per curie by the different components, expressed in gram calories, is as follows: alpha radiations and recoil atoms, 98.5; beta radiations, 4.3; and gamma radiations 6.5; total 109.3. In the arrangement employed for our experiments the alpha radiations and recoil atoms cannot penetrate the walls of the container in which the emanation is confined. Some low velocity beta radiations also fail to penetrate the walls of the container. In any experiment then we have available less than 10.8 gram calories per hour, per curie. Unfortunately, in the paper referred to in foot-note 4 these values are not correctly stated.

Hence it follows that the time rate of change in the concentration of active enzyme is proportional to the concentration of the active enzyme and the *activity* or *power* of the radium emanation.

CONCLUSION.

In this communication we have introduced a unit to express *activity* or *power* of a given preparation of radium emanation. We have named this unit the *curie-power* and defined it as *the activity of 1 curie of radium emanation and its radioactive products in equilibrium with it.* We suggest the introduction of this unit in order that we may make a more comprehensive statement of our experimental observation.

In the radiochemical reaction effected by the radiations (beta and gamma) from radium emanation in which enzymes are inactivated the chemical change in a given system is a function of the product of two variables; namely, the average activity of the radium emanation, P_a, expressed in terms of the unit *millicurie-power*, and time, t, expressed in hours. This product has the dimensions of energy and is identical with W which is measured in terms of the energy unit, *millicurie-power hours.*

THE MINIMUM CONCENTRATION OF OXYGEN FOR LUMINESCENCE BY LUMINOUS BACTERIA.

By E. NEWTON HARVEY and THOMAS F. MORRISON.

(*From the Physiological Laboratory, Princeton University, Princeton.*)

(Received for publication, July 9, 1923.)

It has been recognized for a long time that luminous organisms require very little oxygen for light production, so little in fact that some observers have declared the luminescence to occur without oxygen.[1] Although it is sometimes difficult to free a solution of a luminescent substance from the last traces of oxygen, and this fact is responsible for the controversy, it is safe to say that all bioluminescence is dependent on dissolved oxygen.[2]

This statement is easily verified, as far as luminous bacteria are concerned, by allowing an emulsion of luminous bacteria in sea water to stand in a test-tube. In a short time, depending on the number of bacteria present, the tube, formerly glowing throughout, becomes perfectly dark except at the surface in contact with air. The bacteria have used up all the oxygen in the sea water. Beijerinck[3] employed this method to demonstrate the activity of chloroplasts in decomposing CO_2, apart from the living plant cell. A mixture of luminous bacteria and thoroughly ground clover leaves containing suspended chloroplasts was allowed to stand in perfect darkness until all the oxygen dissolved in the emulsion had been used up, and the tube became dark. If a match were now lighted, the light, striking the chloroplasts, caused them to decompose CO_2, as during photosynthesis, with consequent liberation of oxygen. The oxygen formed then caused the luminous bacteria to glow for a short time, until again used up.

In the above experiment we employ luminous bacteria as a test for oxygen and it is proper to inquire what concentration of oxygen

[1] Kanda, S., *Am. J. Physiol.*, 1919–20, l, 544.

[2] Harvey, E. N., *Am. J. Physiol.*, 1920, li, 580.

[3] Beijerinck, M. W., *Kon. akad. van wetensch. te Amsterdam*, 1902, iv, 45.

can be detected in this way. It is quite obvious that in any determination of such a small concentration of oxygen, (1) the emulsion of luminous bacteria must be continually maintained in equilibrium with some inert gas containing so small a percentage of oxygen that a just perceptible light is produced; (2) the concentration of luminous bacteria must be sufficiently small so that the continuous utilization of oxygen by the bacteria does not disturb the equilibrium. These two conditions have been realized in the experiments described in this paper. The continual maintenance of equilibrium is easily obtained by rapid bubbling of the gas mixture through the bacterial emulsion which was well below the concentration, as determined by experiment, at which an appreciable amount of oxygen was used.

The method of mixing gases by means of flow-meters, so widely used during the war, was employed. The apparatus is shown in Fig. 1. From a tank of electrolytic hydrogen, hydrogen gas, containing 0.0034 by volume oxygen (as determined by analysis), passed through a Y-tube to two flow-meters, *A* and *B*. The gas which passed through the *A* flow-meter, giving a rapid flow, had all of its oxygen removed by passing through a quartz tube, *C*, over platinized asbestos[4] heated to dull redness, and was absolutely pure hydrogen,[5] so far as oxygen was concerned. The gas passing the *B* flow-meter, which contained a very fine capillary, and hence a slow flow of gas, contained the same amount of oxygen as that in the tank, 0.0034. The gases from *A* and *B* were mixed at the three-way cock, *D*, and passed through the bacterial emulsion in the tube, *E*. A safety trap at *F* and needle valves at the tank and at *G* to regulate the flow of gas, completed the arrangements. All parts of the apparatus train were glass joints or connections of lead tubing with de Khotinsky cement, the only rubber connection being a thick short tube between *D* and *E*, and another making connection with the hydrogen tank *before* the hydrogen passed over the platinized asbestos. It is

[4] In some experiments a platinum spiral heated red hot by passing an electric current through it was substituted for the platinized asbestos. Both gave efficient oxygen removal.

[5] Dr. H. S. Taylor of Princeton University has very kindly calculated for us that hydrogen so treated would contain oxygen at a pressure of 2×10^{-26} atmospheres, a negligible quantity.

not possible to keep pure hydrogen free of oxygen if it is carried through any distance of rubber tubing. In order to prevent contamination of pure hydrogen with oxygen dissolved in the manometer fluids the *A* flow-meter manometer, after careful washing out with pure hydrogen, was filled with alkaline pyrogallol solution from *H*, while the *B* flow-meter manometer contained water in equilibrium with hydrogen containing 0.0034 oxygen, filled from *J*.

Once a flow-meter has been calibrated for a given gas in terms of the difference in pressure at the two ends of the capillary, it can be used at any time for the same gas and with the same manometer fluid. Calibration of *A* and *B* was effected by determining the time necessary for a given volume of hydrogen gas to pass through when the difference in pressure at the ends of the capillary was varied, and a curve drawn relating cubic centimeter flow per second to pressure difference. Rate of flow through *A* varied in the range of pressure differences actually used from 2.53 cc. per second at 14 cm. to 3.17 cc. per second at 18 cm. alkaline pyrogallol solution. Rate of flow through *B* varied from 0.006055 cc. per second at 24 mm. to 0.007888 cc. per second at 32 mm. water.

The percentage of oxygen in the hydrogen was determined by passing the gas through a long tube of $CaCl_2$ to dry it, then over hot platinized asbestos which formed water, by combination of any oxygen with the hydrogen. The gas then passed through two small U-tubes filled with $CaCl_2$ to collect the water formed, and was measured over water in a collecting flask. The second $CaCl_2$ U-tube was not necessary as weighings showed no increase in weight. All the water was absorbed by the first tube. The second tube served as a control. 2400 cc. of gas at 18°C. and 767 mm. Hg gave 0.0122 gm. water, an average of three determinations within 0.1 mg. of each other. Reduced to 0°C. and 760 mm. pressure and corrected for the vapor tension of water at 18°C., 2227 cc. of hydrogen contained 7.589 cc. of oxygen at 0°C. and 760 mm. or 0.0034, 0.34 per cent.

An actual determination of the oxygen necessary for luminescence is run somewhat as follows: After flushing out the whole apparatus with tank hydrogen through its various exits, the quartz tube, *C*, with its platinized asbestos is heated and *A*, as well as vessel *H*,

containing alkaline pyrogallol, flushed out with oxygen-free hydrogen. The alkaline pyrogallol is then allowed to flow into the arms of the manometer tube. The water in *J* is then brought into equilibrium with tank hydrogen by shaking and refilling with tank hydrogen several times and then allowed to flow into the manometer of the *B* flow-meter. The emulsion of luminous bacteria[6] in sea water is placed in *E* which is then attached to the three-way cock, *D*, so turned that pure hydrogen gas alone passes through the bacteria. The flow is regulated by turning the needle valve at the hydrogen tank to give a flow of gas, corresponding to a difference in level of about 16.7 cm. alkaline pyrogallol solution. With needle valve, *G*, shut so that no flow of gas passes the *B* flow-meter, the bacteria in *E* are absolutely dark. Observations must be made in a black box in a dark room to prevent confusion from reflected light, and the eyes perfectly dark adapted. Upon now opening needle valve, *G* very slowly to admit more and more tank hydrogen and hence a little oxygen, the bacteria just begin to glow at approximately 25 mm.[7] water difference of pressure of *B*, corresponding to 0.00630 cc. per second. The level in the *A* flow-meter will have changed slightly perhaps to 16.6 cm., corresponding to 3.00847 cc. per second. There is thus passing through the bacterial emulsion 3.01477 cc. of gas each second, of which $\frac{0.00630}{3.01477}$ is supplied by the *B* flow-meter and of this gas supplied by the *B* flow-meter 0.0034, or 0.34 per cent, is oxygen. A simple calculation shows that in the gas flowing through the luminous bacteria there is 0.0000071 oxygen, or 0.0007 per cent. This corresponds to one part oxygen in 143,000 parts hydrogen by volume, or 0.0053 mm. Hg oxygen pressure. Assuming that Henry's law holds, since 1 cc. of sea water dissolves about 0.027 cc. of oxygen[8] at 20°C. and 760 mm. Hg there will be present in each cubic centimeter of the sea water containing luminous bacteria glowing very

[6] The luminous bacteria were isolated from fish obtained at the fish store in Princeton and grown in sea water containing 1 per cent Bacto-peptone, 1 per cent glycerol, some beef extract, and adjusted to a pH of 7.7.

[7] Average of eight determinations.

[8] Fox, *Tr. Farad. Soc.* 1909, v, 77.

faintly at 0.0053 mm. Hg oxygen pressure, 0.00000019 cc. of oxygen or about 0.00000000027 gm. of oxygen, or 1 part oxygen by weight in 3,700,000,000 cc. of sea water.

2 mm. change of level in *B* downward from 25 to 23 mm. Hg will cause the light in *E* to disappear, while 2 mm. change of level of *B* upward from 25 to 27 mm. Hg, corresponding to 0.00677 cc. per second, giving the bacteria 0.0000076 oxygen, will cause an unmistakably brighter light to appear in *E*. Hence the method is sensitive to 5 parts in 76 or about 6 per cent and the greatest error is no doubt the variation in sensitiveness of the dark adapted eye or variations in the light of the bacteria. A number of the values

TABLE I.

Readings of Flow-meters When Bacteria Give Just Perceptible Light.

Experiment No.	*A* manometer.	Flow through *A* per sec.	*B* manometer.	Flow through *B* per sec.
	mm.	cc.	mm.	cc.
1	148	2.689	23	0.00583
2	145	2.643	23	0.00583
3*	145	2.643	28	0.00700
4	176	3.141	21	
5	176	3.141	24	
6	176	3.141	24 = 24.6	0.00621
7	176	3.141	28	
8	176	3.141	26	

* This determination works out to 0.0000089 oxygen.

obtained at different times with the same or with different emulsions of luminous bacteria are shown in Table I.

An experiment to show the values obtained with different concentrations of bacterial emulsions, run the same evening on the same bacteria is given in Table II. C represents a fairly concentrated bacterial emulsion which was diluted with sea water to give C/20, C/30, etc. It will be noted that with concentrated bacterial emulsions more oxygen is necessary to give a just visible luminescence, as shown by the values of the *B* flow-meter, because the bacteria are present in sufficient quantity to use up some of that supplied. However, from a dilution of C/30 to C/1,000 we have practically con-

stant readings for *B* which indicates that concentration of bacteria is playing no part in these determinations. The last eight values given in the table are the ones averaged for calculation of the value 0.000007 oxygen.

TABLE II.

Readings of Flow-meters When Bacteria of Varied Concentrations Give Just Perceptible Light.

Concentration of bacterial emulsion.	*A* manometer.	Flow through *A* per sec.	*B* manometer.	Flow through *B* per sec.
	mm.	*cc.*	*mm.*	*cc.*
C	165	2.983	47	0.01130
	165	2.983	49 = 48	
C/20	165	2.983	31	0.00722
	165	2.983	31 = 31	
C/30	165	2.983	24	0.00630
	165	2.983	26 = 25	
	166	3.008	24	
	166	3.008	25	
C/100	166	3.008	26	0.00630
	166	3.008	24 = 25	
C/500	166	3.008	26	
	166	3.008	25	
C/1,000	166	3.008	26	
	166	3.008	24	

SUMMARY.

A method is described for measuring the concentration of oxygen to allow just perceptible luminescence of luminous bacteria. The value turns out to be extraordinarily low, about 0.005 mm. Hg pressure O_2 or 1 part by weight oxygen dissolved in 3,700,000,000 cc. sea water.

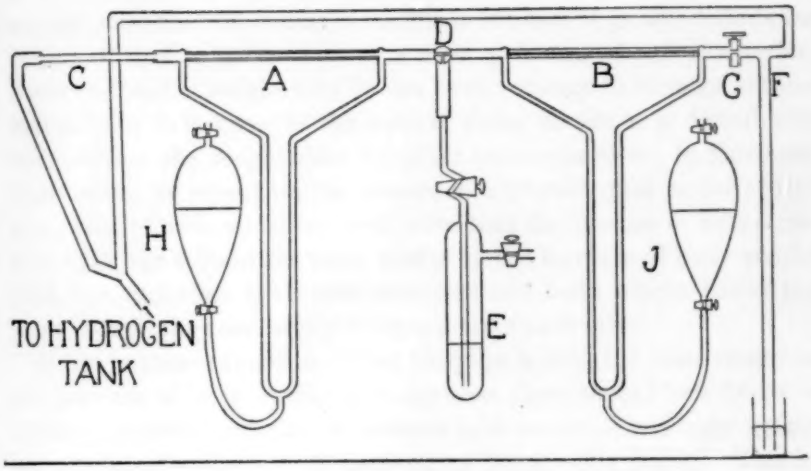

FIG. 1.

THE RATE OF GROWTH OF THE DAIRY COW.
III. The Relation Between Growth in Weight and Increase of Milk Secretion with Age.

By SAMUEL BRODY, ARTHUR C. RAGSDALE, and CHARLES W. TURNER.

(*From the Department of Dairy Husbandry, University of Missouri, Columbia.*)

(Received for publication, June 29, 1923.)

In a preceding communication[1] it was shown that after the age of 2 years, the rate of growth of the dairy cow declines in a non-cyclic manner, and that the course of decline in the rate of growth follows the course of decline in the rate of a monomolecular chemical reaction. Since increase in weight may be due to the storage of inert substances rather than to increase in the mass of living tissues, it is desirable to substantiate the weight data by other measurements. In this communication we report on the change of a physiological activity with age—that of milk secretion—and show that the increase of milk secretion with age follows the same course as the increase of body weight with age, and that while milk secretion and body weight follow the same course, they are largely independent of each other.

If the increase of milk secretion with age follows the same course as the increase of body weight with age then there should be a linear or directly proportional relation between milk secretion and body weight for a group of animals of all ages during the growing period. This is, in fact, the case as shown in Fig. 1 plotted from the data in Table I. The data is confined to the age interval of from 2 years, the usual age when milking begins, to 9 years, the age of maximum milk secretion and body weight of the dairy cow.

It is of interest to fit an equation to the data in Fig. 1 in order to determine whether the relation between body weight and milk secretion with age is an expression of a general law which will apply below the range of observation of 2 years. Fitting an equation of the first degree to the data, we obtain

$$F = 1.0425W - 472.32$$

[1] Brody, S., Ragsdale, A. C., and Turner, C. W., *J. Gen. Physiol.*, 1922–23, v, 445.

in which F is the yearly fat production for any body weight, W. That is, after the animal reaches the body weight of 472 pounds there is an increase of 1.0425 pounds in milk fat production per year, for an increase of one pound of body weight with age. Since the animal is about 13 months of age when she weighs 472 pounds,[2] then if this

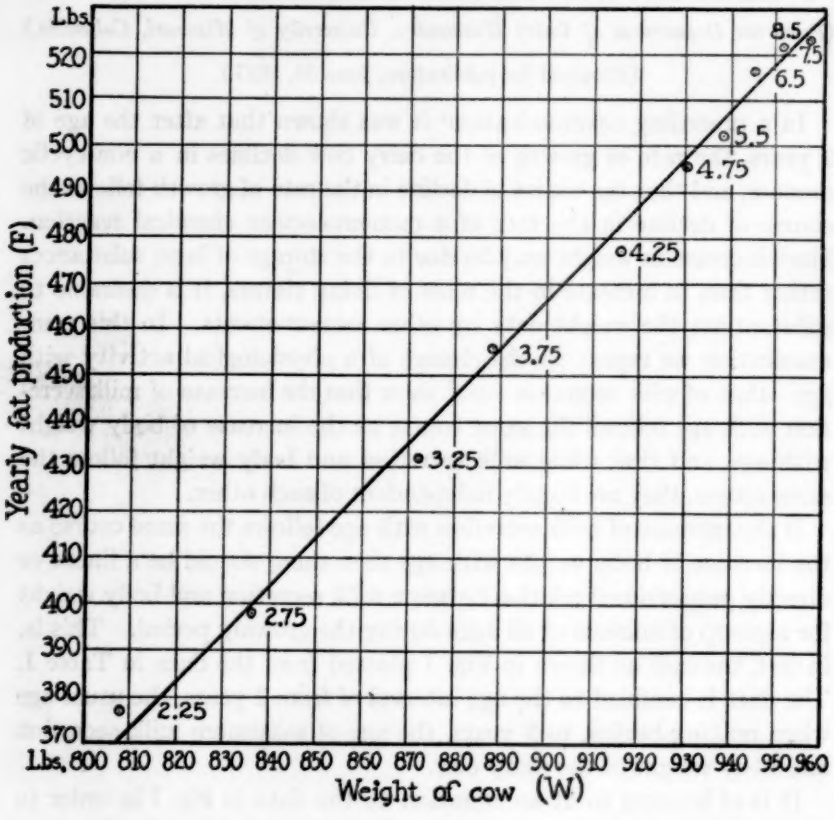

FIG. 1. The increase of milk secretion with increasing body weight with age in Jersey cattle. The smooth line passing through the observed values was plotted from the equation $F = 1.0423W - 472.32$ in which F is the yearly milk fat production for any body weight (W). From this equation an increase of 100 pounds in the weight of the body with age is accompanied by an increase of about 104 pounds of milk fat per year. The numerals on the curve represent the ages of the animals in years.

[2] *Cf.* Eckles, C. H., *Univ. Missouri Agric. Exp. Station, Research Bull. 36*, 1920.

equation is an expression of a general law, the dairy cow should be able to begin secreting milk at a little over one year of age. This is in agreement with experience. Calves have been known to conceive as early as 5 months of age, and to calve at 14 months of age. This equation therefore appears to be rational, and incidentally seems to indicate that there is no material development of mammary milk secreting tissue until the age of sexual maturity, or at any rate, until the age of adolescence is reached.

TABLE I.

*The Increase of Body Weight and Milk Secretion with Age in the Dairy Cow.**

Age.	Body weight.		Butter fat production per year.										
	Jersey.		Jersey.		Shorthorn.		Ayrshire.		Guernsey.		Holstein.		
	No. of cows included.	Average weight.	No. of cows included.	Average production.	No. of cows included.	Average production.	No. of cows included.	Average production.	No. of cows included.	Average production.	Age.	No. of cows included.	Average production.
yrs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*		*lbs.*	*yrs.*		*lbs.*
2.25	3155	808	2829	377	123	268	817	333	3559	407	2.5	2454	508
2.75	1449	836	1261	398	183	274	893	366	1682	423	3.5	1523	570
3.25	1523	872	1271	432	82	251	442	367	1374	436	4.5	1238	615
3.75	1122	888	992	456	85	317	461	389	1192	472	5.5	1116	644
4.25	1171	916	978	477	61	348	376	408	1093	486	6.5	836	666
4.75	916	930	809	496	64	356	340	430	884	501	7.5	583	665
5.5	1692	938	1487	502	70	376	545	448	1133	511	8.5	396	657
6.5	1235	945	1067	516	80	383	399	455	897	521			
7.5	965	952	837	521	66	405	298	478	572	528			
8.5	621	957	565	523	65	402	225	453	369	527			

* Compiled from the records of Register of Merit Jersey, Record of Merit Shorthorn, Advanced Register Ayrshire, Guernsey and Holstein cattle.

While the linear relation between increase in body weight and milk secretion with age in Fig. 1 definitely shows that growth in body weight and increase in milk secretion with age follow the same course, it is useful to bring out this relation by pointing to the parallelism between the two processes. In Fig. 2 the curves of increase in body weight (broken curve) and milk secretion (continuous curve) of the Jersey cow are parallel and both follow the same exponential equation

$$X = A\left(1 - e^{-kt}\right)$$

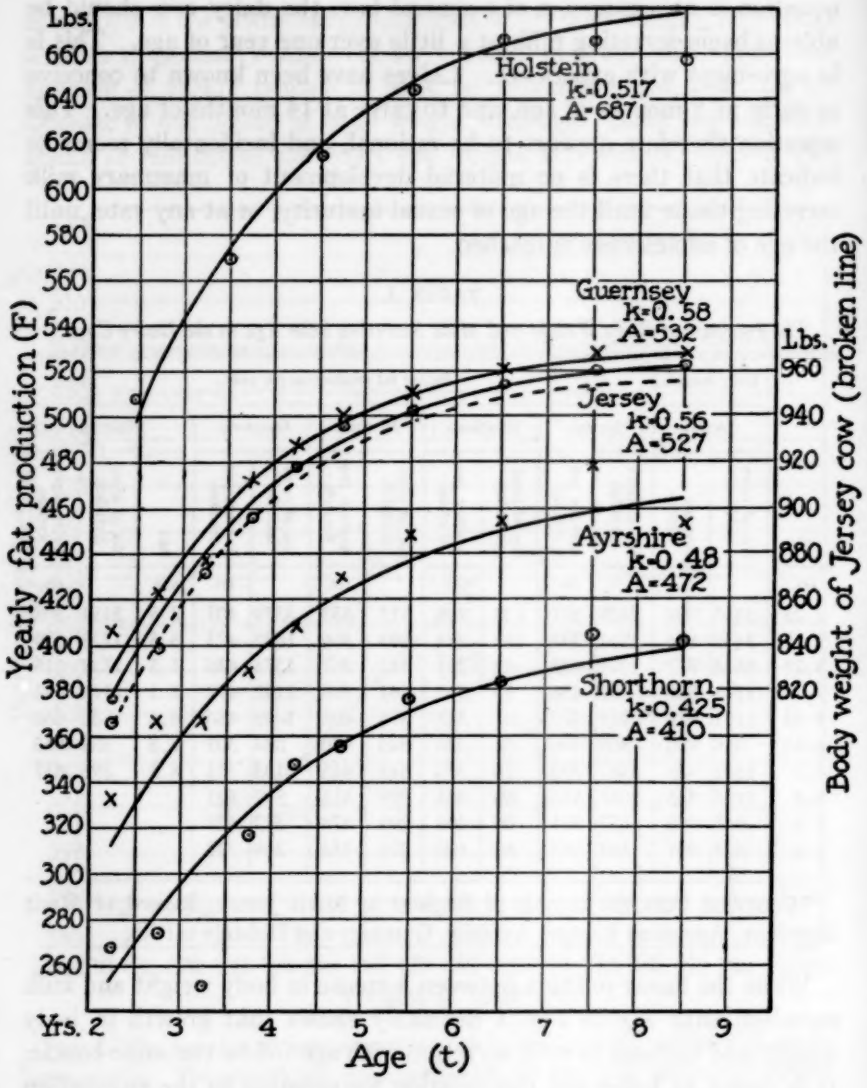

FIG. 2. The increase of milk secretion with age in the dairy cow. The smooth curves passing through the observed values were plotted from the equation $F = A(1-e^{-kt})$ in which F is the yearly fat production at any age (t). The numerical values of the constants A and k for the several breeds are indicated on the curves. The broken curve shows the growth in weight of the Jersey cow.

where X is the body weight, or milk secretion at any age, t, and k is the velocity constant which is of approximately the same numerical value for both curves. This equation also represents the course of milk secretion of other breeds of cattle as shown by the close agreement between the values computed from this equation represented by the smooth curves in Fig. 2, and the observed values.

The data in Figs. 1 and 2 show conclusively that growth in weight, and increase of milk secretion with age follow the same course—the course of a monomolecular chemical reaction—thus further substantiating the theory that growth is limited by a chemical reaction.[3]

[3] The decline in growth with advancing age is often spoken of as due to the dying out of the "growth impulse." From the standpoint of the quantitative interpretation of this equation, the concept of growth impulse may be used in the same sense as the concept of "limiting substance." This equation shows that the growth impulse dies out, or the growth-limiting substance is used up at a constant ratio (or per cent) of itself. This idea is illustrated in the following table on the growth in weight of the Jersey cow, assuming that the mature weight, 960 pounds, is a measure of the limiting substance at the beginning of growth, and that the weight at any age t is a measure of this limiting substance used up to that age t.

Age. (t)	Weight of Jersey cow. (X)	Amount of limiting substance or impulse left, or growth yet to be made. (960-X)	Ratio of each value of (960-X) to the preceding value of (960-X).
yrs.	*lbs.*		
1.75	748	212	
			0.740
2.25	803	157	
			0.738
2.75	844	116	
			0.732
3.25	875	85	
			0.752
3.75	896	64	
			0.734
4.25	913	47	
			0.744
4.75	925	35	

The amount of limiting substance or impulse, at each age is seen to be about 74 per cent of the amount of this substance, or impulse, at the preceding age. In other words, the proportional amount of limiting growth substance, or of growth impulse, that changes in unit time is a constant.

Having determined the fact that the increase of milk secretion with age follows the same course as the increase of body weight with age, the questions arise whether the increase of milk secretion with age is dependent on the increase of body weight (*i.e.*, whether the increase of milk secretion with age is limited by the increase of nutrients circulating in the blood which are roughly proportional to the body weight); or whether the increase of milk secretion with age is dependent on the development of milk secreting tissues, and on other factors consequent to increase in age. These questions may be answered in a general way by separating, as far as practicable, the factors of age and body weight, and determining the effect of each of these factors on milk secretion. A practical, if not an entirely satisfactory[4] method of making a separation between body weight and age is to classify the animals into convenient age groups and determine the relation between the body weight of the animals within the age groups and their milk secretion; also to classify the animals into convenient body weight groups and determine the relation between the age of the animals within the weight groups and their milk secretion. This has been done and the results are shown in Fig. 3 plotted from data in Tables II and III.

Fig. 3 shows the relation between milk secretion and body weight at constant age. Qualitatively, the effect of age is apparent by the fact that each age group has its own curve which is not connected to the curves of other age groups. Quantitatively, the relative contribution of increasing body weight and of age (or rather of factors other than body weight incident to increase of age) on increasing milk secretion with age, may be roughly estimated from a comparison of the slopes of the curves of Figs. 1 and 3. From Fig. 1, showing the relation between milk secretion and body weight with age, an increase in body weight with age by 100 pounds is accompanied by an increase in yearly butter fat production of slightly over 100 pounds. From Fig. 3 showing the relation between body weight and milk secretion at constant age,

[4] This method is not entirely satisfactory because differences in body weight at the same age may be due to widely different causes; some of the causes may be purely genetic, others purely environmental, and still others to different combinations of genetic and environmental factors. These different factors and combinations of factors may differently influence milk secretion at different ages and weights.

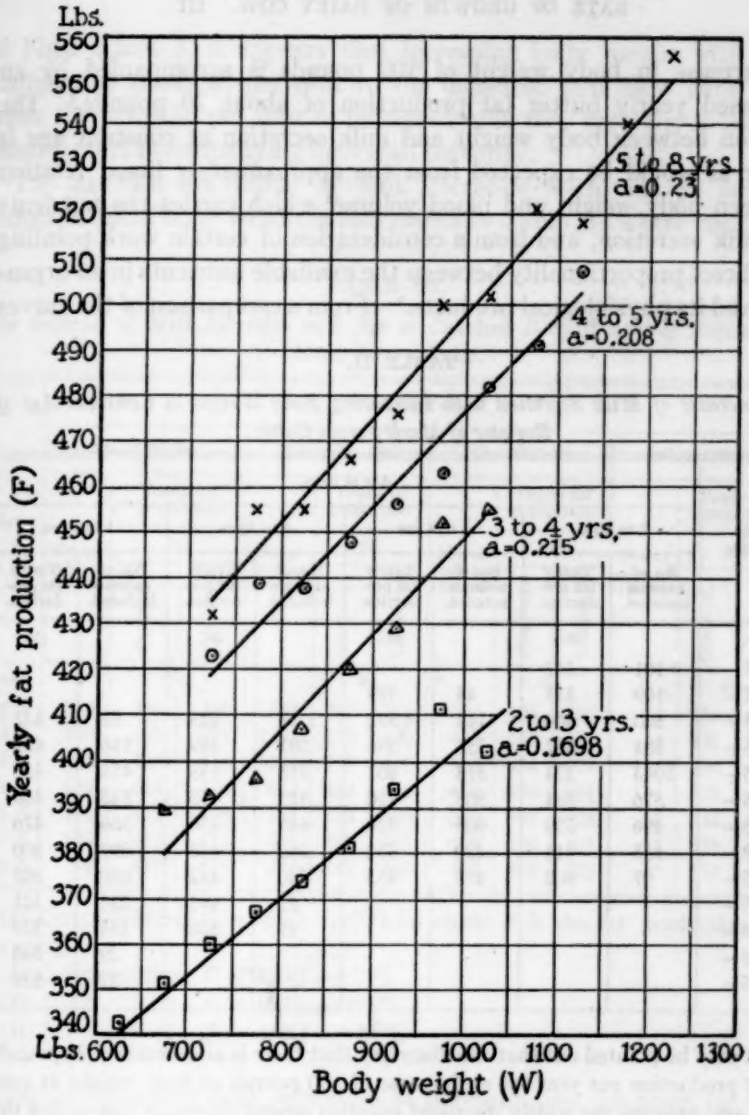

FIG. 3. The increase of milk secretion with increasing body weight at constant age. The smooth lines passing through the observed values were plotted from the equation $F = aW + b$ in which F is the yearly milk fat production for the body weight (W) at the constant ages indicated on the curves; a is the constant increase of yearly fat production for each added pound of body weight. From the values of a indicated on the curves, an increase of 100 pounds in the weight of the body is accompanied by an increase of about 20 pounds of milk fat production per year.

an increase in body weight of 100 pounds is accompanied by an increased yearly butter fat production of about 20 pounds.[5] The relation between body weight and milk secretion at constant age is linear as would be expected from the approximately linear relation between body weight and blood volume[6] which carries the nutrients for milk secretion, and from a consideration of certain work pointing to a direct proportionality between the available nutrients in an organism, and its physiological products.[7] From a comparison of the curves

TABLE II.

The Increase of Milk Secretion with Increasing Body Weight at Constant Age of Register of Merit Jersey Cattle.

Body weight.	Age of cows.							
	2 to 3 yrs.		3 to 4 yrs.		4 to 5 yrs.		5 to 8 yrs.	
	No. of animals included.	Yearly fat production.	No. of animals included.	Yearly fat production.	No. of animals included.	Yearly fat production.	No. of animals included.	Yearly fat production.
lbs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*
625–	101	342						
675–	199	351	43	389				
725–	540	360	142	392	38	423	52	432
775–	584	367	255	396	101	439	156	455
825–	1043	374	573	407	315	438	458	455
875–	576	381	508	420	318	448	543	466
925–	496	394	605	429	445	456	866	476
975–	155	411	220	452	262	463	599	500
1025–	97	402	185	455	240	482	680	502
1075–					82	491	224	521
1125–					49	508	154	518
1175–							29	540
1225–							27	556

[5] It may be pointed out that the discovery that there is an increase of 20 pounds of fat production per year for an increase of 100 pounds of body weight at constant age, answers the widely discussed question among dairymen concerning the relative economy of milk production from heavy and light cows. 20 pounds of butter fat is barely sufficient to cover the cost of maintenance of 100 pounds of body weight per year.

[6] Donaldson, H. H., The rat, Memoir of The Wistar Institute of Anatomy and Biology, No. 6, Philadelphia, 1915, 96.

[7] *Cf.* Loeb, J., *J. Gen. Physiol.*, 1919–20, ii, 297.

of Figs. 1 and 3, it appears that increasing body weight with age contributes about 20 per cent to the increased milk flow with age, while the other 80 per cent of increased milk flow with age is due to other factors accompanying increased maturity.

The fact that the course of milk secretion with age is largely independent of body weight is also shown in Table III where the com-

TABLE III.

The Increase of Milk Secretion with Age at Constant Body Weight of Register of Merit Jersey Cattle.

Age.	Weight of cows.											
	750 to 849 lbs.			850 to 949 lbs.			950 to 1049 lbs.			1050 to 1151 lbs.		
	No. of cows included.	Yearly fat production. (1)		No. of cows included.	Yearly fat production. (2)		No. of cows included.	Yearly fat production. (3)		No. of cows included.	Yearly fat production. (4)	
		Observed.	Calculated.		Observed.	Calculated.		Observed.	Calculated.		Observed.	Calculated.
yrs.		*lbs.*			*lbs.*			*lbs.*			*lbs.*	
2.5	1869	354	346	1289	385	381	259	405	406	23	418	389
3.5	799	401	401	1241	429	430	422	458	453	44	431	449
4.5	446	433	434	1019	458	457	513	474	478	114	496	486
5.5	239	463	454	842	483	472	476	496	491	131	519	507
6.5	174	452	466	563	475	480	503	503	498	97	524	520
7.5	130	470	474	443	487	485	499	499	501	91	529	528
8.5	87	479	478	255	487	487	492	492	503	47	533	533

(1) Computed from $F = 485(1-e^{-0.5t})$ in which F is the fat production for the age (t).

(2) Computed from $F = 490(1-e^{-0.6t})$.

(3) " " $F = 505(1-e^{-0.65t})$.

(4) " " $F = 540(1-e^{-0.51t})$.

puted and observed milk secretions at different ages are given for the groups of animals at constant body weight. The equation of a monomolecular reaction which represents growth in body weight and increase in milk secretion with age shown in Fig. 2, also represents fairly satisfactorily the increase in milk secretion with age at constant body weight shown in Table III.

SUMMARY.

It is shown that from 2 years, the age when milk secretion usually begins, to 9 years, the age of maximum body weight, the increase of milk secretion with age follows the course of growth in body weight—both can be accurately represented by the equation of a monomolecular chemical reaction having a velocity constant of approximately the same numerical value. While increase in milk secretion and increase in body weight with age follow the same course, it is shown that increasing body weight contributes only about 20 per cent to increasing milk secretion with age. The fact that milk secretion and body weight follow the same course, even though they are largely independent of each other indicates that increase in body weight is a good measure of growth of the dairy cow; this fact also shows that the increase of milk secretion with age may be used as a measure of growth. The fact that milk secretion, like body weight, follows the course of a chemical reaction, adds further support to the theory that growth is limited by a chemical reaction.

THE RATE OF GROWTH OF THE DAIRY COW.

IV. Growth and Senescence as Measured by the Rise and Fall of Milk Secretion with Age.

By SAMUEL BRODY, ARTHUR C. RAGSDALE, and CHARLES W. TURNER.

(From the Department of Dairy Husbandry, University of Missouri, Columbia.)

(Received for publication, July 17, 1923.)

The increase of milk secretion and body weight in the dairy cow follows exactly the same course from the age when milk secretion usually begins (2 years), to the age when maximum body weight is reached (about 8 years).[1] It is therefore concluded that the increase of milk secretion with age may be used as a measure of growth in the same sense that the increase of body weight with age is used as a measure of growth. After the age of maximum body weight is reached, it remains practically constant and can therefore no longer be used to measure the effect of age on the condition of the body; but milk secretion takes a downward course after passing the age of maximum body weight and steadily declines with age. This steady decline of milk secretion with age suggests the possibility of using milk secretion as a measure of the effect of age on the body after age ceases to have an appreciable effect on body weight—in other words, to use the declining curve of milk secretion as a measure of senescence. In the absence of contradictory evidence, there seems no reason to believe why the course of milk secretion which was found to be a good quantitative measure of growth during the growing phase of life, should not also be a good quantitative measure of senescence during the declining phase of life. Adopting the point of view that the rising and falling curve of milk secretion with age is a representation of developing and declining physical powers with age due to the processes classed under growth and senescence, we have brought together a large amount of data on the change of milk secretion with

[1] Brody, S., Ragsdale, A. C., and Turner, C. W., *J. Gen. Physiol.*, 1923–24, vi, 21.

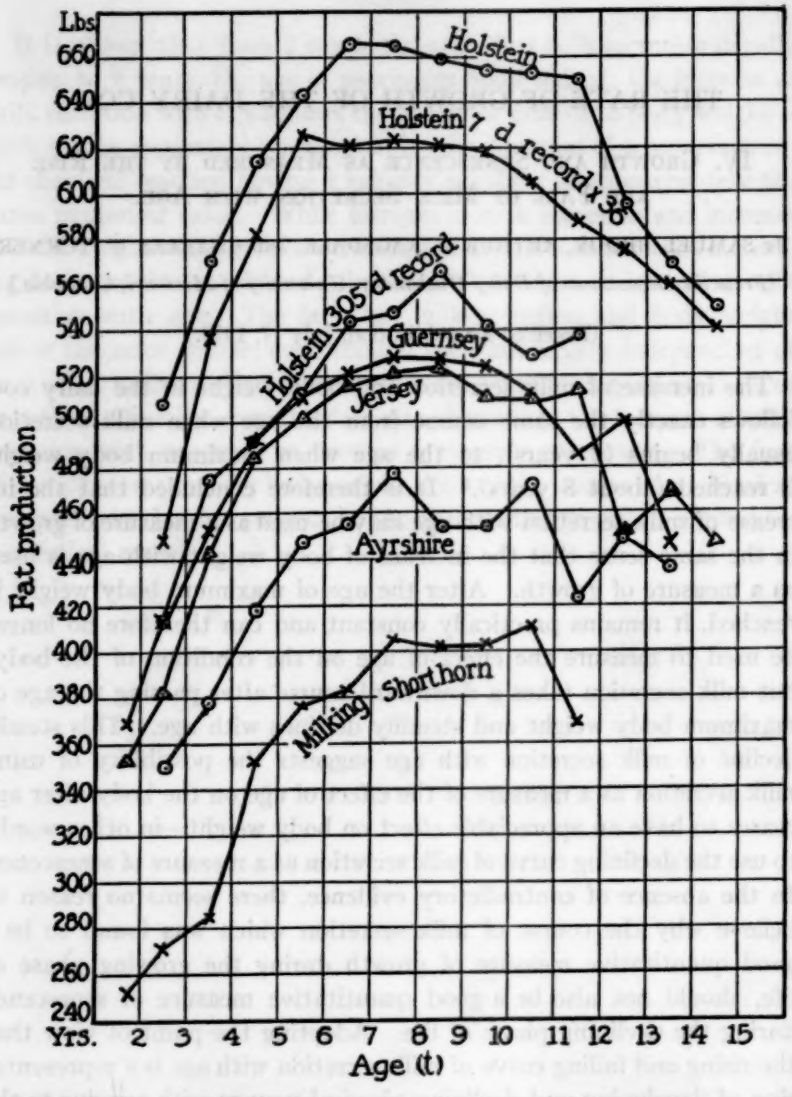

FIG. 1. The course of milk secretion with age for several breeds of dairy cattle plotted from data in Table I. With the exceptions noted on the curves, the curves represent the butter fat production per year of 365 days. For the Guernsey cows, the records represent 365 days or less, if the cow happened to dry off before the end of the year. The curves in this figure represent a total of 150,544 records.

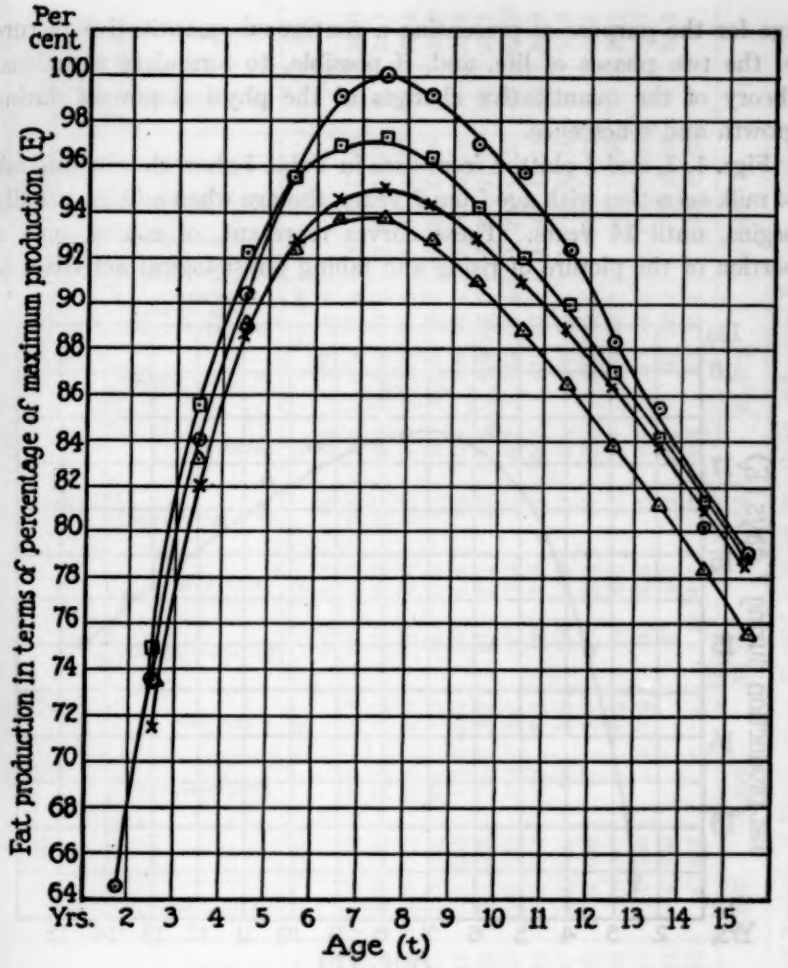

FIG. 2. Comparison between computed and observed values of milk secretion with age for yearly records. The circles represent observed values of the weighted averages of all cows yearly and 305 day records expressed as percentage of maximum production given in table I. The other values were obtained from trial equations as follows: ⊡ represents $F_t = 142.4e^{-0.377t} - 126.15e^{-0.331t}$; + represents $F_t = 142.4e^{-0.0377t} - 126.15^{-0.310t}$; △ represents $F_t = 142.4e^{-0.04t} - 120e^{-0.310t}$. After the age of 12 years, the observed values are not reliable due to the small number of animals represented. The observed values in this figure are based upon 45,984 yearly and 10 monthly records.

age for the purpose of presenting a continuous quantitative picture of the two phases of life, and, if possible, to formulate a rational theory of the quantitative changes of the physical powers during growth and senescence.

Figs. 1, 2, and 3 plotted from data in Table I show the rise and fall of milk secretion with age from 2 years, the age when milking usually begins, until 14 years. These curves represent, of course, only a portion of the picture of rising and falling physiological activities of

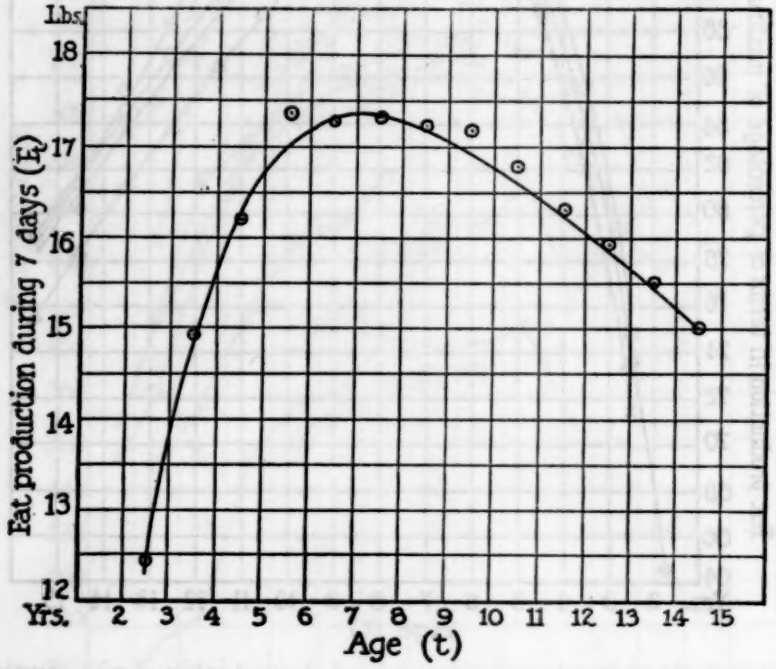

FIG. 3. Comparison between computed and observed values of milk secretion with age for 7 day records given in Table I. The circles represent observed values, the smooth line represents the equation $F = 22.1(e^{-0.026t} - 1.2e^{-0.46t})$. The observed values in this figure are based on a total of 104,560 records. The rate of senescence, it may be noted, is less steep than the rate of growth. The relative slopes of the two sides of the curve at any ages t and t_2, may be found by substituting the ages for t in the equation $\frac{dF}{dt} = 22.1(-0.026e^{-0.026t} + 1.2 \times 0.46e^{-0.46t})$.

TABLE I.

*Data on the Course of Milk Secretion with Age in the Dairy Cow.**

Age.	Ayrshire cows.		Guernsey cows.		Holstein cows. 365 day records.		Holstein cows. 305 day records.		Jersey cows.		Milking Shorthorn cows.		Weighted average of all cows.		7 day records, Holstein cows.		7 month records of a group of longlived cows.	
	No. of cows included.	Fat per yr.	No. of cows included.	Fat per yr.	No. of cows included.	Fat 365 days.	No. of cows included.	Fat 305 days.	No. of cows included.	Fat 365 days.	No. of cows included.	Fat per yr.	No. of cows included.	Fat expressed as per cent of maximum production.	No. of cows included.	Fat 7 days.	No. of cows included.	Fat per 7 mos.
yrs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*		*lbs.*		*lbs.*		*per cent*		*lbs.*		*lbs.*
1.7			313	368					947	353	15	252	1,275	64.6				
2.5	1,710	350	5,241	412	2,454	508	1,200	413	4,090	383	306	272	15,001	73.6	33,765	12.46	21	24.0
3.5	903	378	2,566	452	1,523	570	762	475	2,263	443	167	285	8,184	84.0	22,019	14.91	24	28.1
4.5	716	418	1,977	492	1,238	615	606	493	1,687	486	125	352	6,349	90.4	16,374	16.20	33	33.1
5.5	545	448	1,133	511	1,116	644	467	513	1,487	502	75	376	4,823	95.5	11,259	17.39	33	35.0
6.5	399	455	897	521	835	666	331	544	1,067	516	80	383	3,609	99.1	8,356	17.26	37	37.4
7.5	298	478	572	528	583	665	223	547	837	521	66	405	2,579	100.0	5,586	17.32	32	37.1
8.5	225	453	369	527	396	659	156	566	565	524	65	402	1,776	99.1	3,256	17.25	40	38.7
9.5	155	454	264	524	232	654	72	541	355	510	43	403	1,121	97.0	1,862	17.19	39	36.9
10.5	100	472	123	512	111	652	46	529	200	509	29	411	609	95.8	1,054	16.80	39	37.9
11.5	52	423	76	486	59	650	17	538	108	513	21	369	333	92.4	548	16.34	39	33.8
12.5	26	450	32	500	37	595	13	441	58	453	13	397	179	88.3	285	15.96	32	32.1
13.5	15	436	24	447	11	569	6	510	31	470	4	399	91	85.5	130	15.54	18	33.3
14.5	8	375	5	491	4	550			13	447	3	360	33	80.2	42	15.04	15	34.8
15.5	4	392	4	446	4	475			8	451	2	353	22	79.1	24	15.40		
Total†	5,156		13,596		8,603		3,899		13,716		1,014		45,984		104,560			

* Compiled from the records of Register of Merit Jersey, Record of Merit Shorthorn, Advanced Register Ayrshire, Guernsey, and Holstein cattle.

† Total number of cows included in this table is 150,544 (exclusive of longlived cows).

this animal. The natural duration of life of the cow is said to be about 30 years. Milk records above 15 years are, however, very scarce because in addition to the accidental deaths which leave relatively few animals by the time this age is reached, the animals are also purposely disposed of due to the fact that they become increasingly unprofitable milk producers with increasing age caused by such factors as the increasing difficulty of breeding with age and decaying teeth.

While these curves represent only about half of the whole life curve, they are nevertheless of value because they show the trend of relative rates of growth and of senescence, and the age of the statistical equilibrium between these two processes. Besides, if the growth and senescence curves continue an unchanged course beyond the range of observation, as is probable, then it should be possible to extrapolate the curves by the use of some suitable formula, thereby completing the whole picture.

The trend of the curves of growth and of senescence having been determined on the basis of an extensive amount of data (there is no doubt that the data presented here are by far the largest amount that was ever brought together on the quantitative variation of milk secretion with age, and with the exception of vital statistics on man, they are the largest body of quantitative data on the change of any physiological activity with age), the next step is to formulate a mathematical expression which would not only represent the empirical curve, but what is far more important, an expression which should be rational in the sense that it is derived theoretically as a conclusion from a general law of nature, and which should be capable of explaining the mechanism of the process under investigation. Pearl and his coworkers[2] found that the expression

$$y = a + cx^2 + d \log x$$

in which y is the milk production and x is the age, may be accurately fitted to the variation of milk secretion with age. However, since

[2] Pearl, R., and Patterson, S. W., *Maine Agric. Exp. Station, Bull. 262*, 1917. Pearl, R., and Miner, J. R., *J. Agric. Research*, 1919, xvii, 285. Pearl, R., Gowen, J. W., and Miner, J. R., *Ann. Rep. Maine Agric. Exp. Station 1919*, 1919, 89. Gowen, J. W., *Genetics*, 1920, v, 111. Gowen, J. W., *Ann. Rep. Maine Agric. Exp. Station 1920*, 1920, 185.

Pearl and his coworkers do not state that this equation explains the mechanism of this variation of the activity of the mammary gland with age, or that the equation was derived as a conclusion from a general law then this is probably an empirical equation and it does not solve the problem of formulating a rational equation explaining the mechanism of the peculiar course of growth and of senescence.

The following theory is suggested as a basis for formulating a rational equation to represent the rising and falling curve of physiological activities with age and therefore as a basis for a quantitative theory of growth and senescence. It is assumed that growth and senescence go on simultaneously from the beginning to the end of life, but that the ratio between the velocities of these two processes varies in a continuous manner throughout life. At the beginning of life the ratio of growth to senescence is infinitely great, while at the time of natural death, at the extreme old age, the ratio of senescence to growth is infinitely great. At the age of maximum, or prime physical development the two processes just balance each other. The view is also adopted that growth and senescence are physicochemical processes governed by the laws of mass action—a view due to Loeb and his coworkers.[3] If growth and senescence are physicochemical processes, then the course of each, growth and senescence, should follow the course of some chemical reaction, since it is a general principle of chemistry that in a system of chemical reactions which are interdependent, the slowest reaction determines the rate of the resultant process,[4] and therefore growth and senescence while complicated processes should theoretically follow the course of chemical reactions of a simple order; and if growth and senescence go on simultaneously, but with a continuously changing ratio of velocities, then it should be possible to express the course of the whole curve of growth and senescence by the mathematical expression which represents the course of simultaneous consecutive chemical reactions. According to this theory, life may be represented symbolically by the expression $X \rightarrow Y \rightarrow Z$ in which $X \rightarrow Y$ is the process of

[3] *Cf.* (*inter alia*) Loeb, J., *Biochem. Z.*, 1906, i, 183. Loeb, J., and Lewis, W. H., *Am. J. Physiol.*, 1901–02, vi, 305. Loeb, J., and Northrop, J. H., *Proc. Nat. Acad. Sc.*, 1916, ii, 456; 1917, iii, 382. Loeb, J., *Scient. Monthly*, 1919, ix, 578.

[4] *Cf.* Walker, J., *Proc. Roy. Soc. Edinburgh*, 1897–98, xxii, 22.

growth which follows the course of a chemical reaction, or reactions, as we have indeed found in studying the growth of the dairy cow,[5] the animal under consideration, and as was found to be the case for the growth of many other organisms;[6] Y→ Z, the process of senescence which it is assumed also follows the course of, and is limited by some chemical reaction. The whole process X→ Z can therefore probably be represented by the equation of some consecutive reactions; for example,

$$M_t = A\left(ae^{-k_1 t} - be^{-k_2 t}\right) \qquad (1)$$

in which k_1 and k_2 are the velocity constants respectively of senescence and growth, M_t, the milk production (or other index of physiological activity with age) at the age, t, and e, the base of natural logarithms.[7]

This equation (1) was in fact found to represent the course of milk secretion with age quite satisfactorily as may be seen in Figs. 2 and 3 where the observed values are fairly close to the values computed from this equation. This equation cannot be rigidly tested, first, because the theory of consecutive reactions is incomplete; second, because since growth and senescence are simultaneous, it is not possible to determine separately the values of the velocity constants k_1 and k_2. The satisfactory fit of the trial equation to the data is, however, extremely suggestive. The oxidation of phosphorous acid by potassium persulfate in the presence of hydriodic acid, is a classical example of consecutive simultaneous reactions. The numerical values of the velocity constants for this reaction were determined separately for the constituent reactions, the reduction of the persulfate by hydriodic acid, and the oxidation of phosphorous acid by iodine. The comparison between the theoretical and observed

[5] Brody, S., and Ragsdale, A. C., *J. Gen. Physiol.*, 1920–21, iii, 623. Brody, S., Ragsdale, A. C., and Turner, C. W., *J. Gen. Physiol.*, 1923–24, v, 445.

[6] *Cf.* (*inter alia*) Robertson, T. B., Principles of biochemistry, for students of medicine, agriculture, and related sciences, Philadelphia and New York, 1920.

[7] For derivation and application of this equation to certain life processes in plants *cf.* Osterhout, W. J. V., Injury, recovery, and death, in relation to conductivity and permeability, Monograph on Experimental Biology, Philadelphia and London, 1922.

values of this reaction *in vitro*[8] and the values obtained from trial equations and observed values in Fig. 2 and especially in Fig. 3 in the variation of milk secretion with age is not unfavorable to the latter.

The fit of the computed to the observed values of milk secretion in Fig. 1 is especially satisfactory considering some of the defects of the observed data. The most serious defect is that the population rapidly decreases with age due to the natural and purposeful elimination of the less fit animals, either because of defective physical vigor causing death at an early age, or because of unsatisfactory milk production. As a result of this elimination, and the elimination due to the minimum entrance requirements for Advanced Registry, only the better animals survive at the more advanced ages, amounting to a comparison between relatively good animals at the more advanced ages with relatively mediocre animals at the earlier ages. This would tend to a relatively higher production at the more advanced ages which may explain the fact shown in Fig. 2 that at later ages the observed production is above the computed production. The increasing difficulty of breeding animals with increasing age resulting in a longer farrow period may likewise increase the relative milk production at the later ages, since it has been shown[9] that pregnancy appreciably decreases milk production. The 7 day data shown in Fig. 3 are superior to the yearly data of Fig. 2, first, because the effect of delayed breeding due to age is absent since the 7 day records are always made before breeding; second, because the 7 day records are made when the animals are at their best, shortly after calving; and third, and most important, since the period of test is only 7 days the animals can be kept for this brief period under the very best conditions, eliminating the many unfavorable factors which come up during the course of a whole year. These uniform and favorable conditions under which the 7 day records are made therefore represent more nearly the genetic capacity of the animals than the yearly records in which environmental factors are less under control. These

[8] Federlin, W., *Z. physikal. Chem.*, 1902, xli, 565. Lewis, W. C. McC., A system of physical chemistry, London and New York, 1920, 3rd edition, 403, 4.

[9] Brody, S., Ragsdale, A. C., and Turner, C. W., *J. Gen. Physiol.*, 1922-23, v, 777.

facts and the fact that the 7 day averages are based on a very much larger number of animals than the yearly averages, may explain the better agreement between observed and computed values for the 7 day records in Fig. 3 than the yearly records in Fig. 2.

SUMMARY.

Data are presented on the effect of age on milk secretion in the dairy cow. From the age when milk secretion usually begins (2 years) to the age when maximum body weight is reached (about 8 years) increase of milk secretion and increase of body weight with age follow the same exponential course, which is the course of a monomolecular reaction of chemistry. After this age, unlike body weight which remains practically constant, milk secretion declines exponentially, that is, the course of decline follows the course of decline of a monomolecular reaction. The whole course of milk secretion with age was therefore found to follow approximately the course of two simultaneous, consecutive, monomolecular reactions. This is taken to mean that growth and senescence go on simultaneously from the beginning to the end of life, and that each follows an exponential law with age; and therefore perhaps that the course of the two processes are limited by two consecutive chemical reactions.

THE RATE OF SENESCENCE OF THE DOMESTIC FOWL AS MEASURED BY THE DECLINE IN EGG PRODUCTION WITH AGE.

By SAMUEL BRODY, EARL W. HENDERSON, AND H. L. KEMPSTER.

(From the Agricultural Experiment Station, University of Missouri, Columbia.)

(Received for publication, July 17, 1923.)

It is well known among poultrymen that egg production in the domestic fowl declines with age. Indeed, it is the general opinion that the decline is so rapid that after the age of 2, or at most 3 years, a hen is no longer a profitable egg producer, and should therefore be disposed of. The decline in egg production with age may be due to the gradual exhaustion of the oocytes, or to the decline in vigor of the organs or tissues which form the limiting factors in egg production, or to both. In either case, whether it be exhaustion of a limited substance on which the process depends, or whether it be the wearing out of a limiting organ, the process may be classed as senescence, and the course of egg production with age may be used as an index of the course of senescence of the limiting tissues or organs. If this reasoning is correct, then data on the course of decline of egg production with age should be suitable material for testing by one method the theory that senescence is a physicochemical process.

If senescence is a physicochemical process, then the course of senescence should follow the course of some chemical reaction, since it is a general principle of chemistry that in a system of chemical reactions which are interdependent, the slowest reaction determines the rate of the resulting process[1] and therefore senescence, while a complicated process, should roughly follow the course of a chemical reaction of a simple order (the slower the limiting reaction as compared to the other reactions in the system, the closer the agreement between the calculated and observed values). The data on egg production presented in

[1] Walker, J., *Proc. Roy. Soc. Edinburgh*, 1897–98, xxii, 22.

Table I and Fig. 1 were analyzed from this standpoint and they were found to follow the course of the equation

$$E_t = Ae^{-kt} \quad (1)$$

in which E_t is the egg production during any age, t, e is the base of natural logarithms, and A and K are constants. This equation indicates that each year's egg production is, regardless of age, the same per cent of the preceding year's production. Thus in Table I, if any value is

TABLE I.

The Decline in Egg Production with Age in the Domestic Fowl.

Age.	No. of birds included.	Egg production per year. (Nov. 1 to Nov. 1.)	
		Observed.*	Calculated.†
yrs.			
1	222	158	158.5
2	221	140	140.1
3	222	124	123.9
4	222	110	109.6
5	193	95	96.9
6	28	89	85.7
7	27	71	75.7
8	6	63	67.0

* The observed values are averages of two breeds and eight different groups of birds kept under different conditions of management. To save space in this general publication, the individual records, and their significance, are omitted,but they will probably be presented in a poultry journal.

† Calculated from the equation $y = 179.2e^{-0.128t}$ in which y is the yearly egg production at the age, t. The egg production during any year is 88 per cent of the preceding year's production.

divided by the preceding value, the quotient is always .88, that is each year's production is 88 per cent of the preceding year's production. In other words, the percentage decline of egg production with age is constant, or exponential, and the course of senescence of the limiting tissues or organs involved in egg production, may therefore be said to follow an exponential law.

This exponential law represented by equation (1) is the same as the law of monomolecular change in chemistry, thereby substantiating

the suggestion that "the natural duration of life would be in reality the time required to complete a chemical reaction or a series of chemical reactions resulting in the production of toxic compounds sufficient to kill or resulting in the destruction of necessary compounds."[2]

Equation (1) may also be used to throw some light concerning the particular factor that limits egg production. The total number of

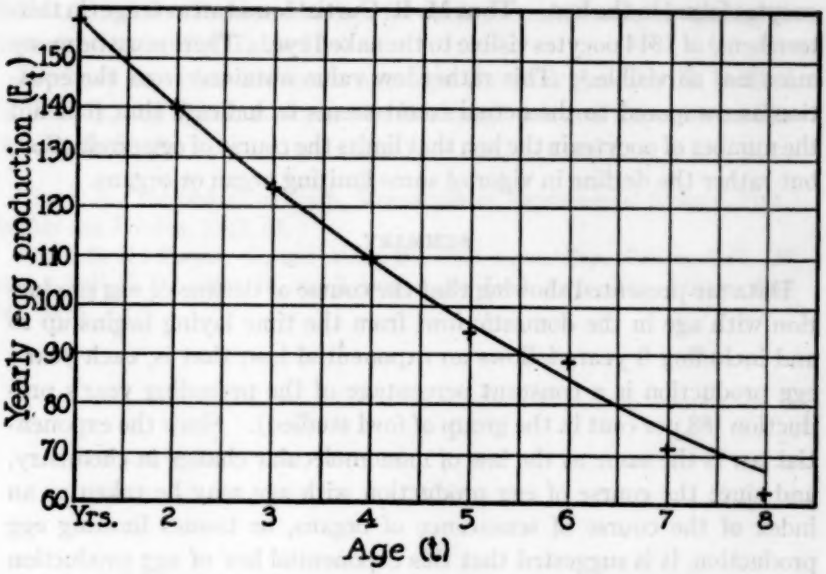

FIG. 1. The decline in egg production with age in the domestic fowl plotted from the averages in Table I. The smooth line represents the computed values; the crosses represent the observed values. The observed values during the 7th and 8th years are not reliable due to the small number of individuals represented in the averages.

eggs the average fowl in Table I would lay if she lived long enough to allow the senescence reaction represented by equation (1) to go to completion would be the total number of eggs laid from the time laying begins ($t = \frac{1}{2}$ year) to the time the senescence reaction is completed (t = infinity). This theoretical number of eggs can be found by

[2] Loeb, J., *Scient. Monthly*, 1919, ix, 578.

integrating equation (1) between the limits of $\frac{1}{2}$ year and infinity as follows:

$$E = \int_{\frac{1}{2}}^{\infty} Ae^{-kt}\,dt = \frac{a}{-k}\left[-e^{-kt}\right]_{\frac{1}{2}}^{\infty} = \frac{179.2}{0.123}\,e^{\frac{-0.123}{2}} = 1369$$

Now this total number of eggs, 1369, that the average hen in Table I can theoretically produce falls considerably below the number of oocytes found in the hen. Thus M. R. Curtis[3] found an average (in thirteen hens) of 1814 oocytes visible to the naked eye. There must be many more not so visible.[4] This rather low value obtained from the equation as compared to the actual count seems to indicate that it is not the number of oocytes in the hen that limits the course of egg production, but rather the decline in vigor of some limiting organ or organs.

SUMMARY.

Data are presented showing that the course of decline of egg production with age in the domestic fowl from the time laying begins up to and including 8 years follows an exponential law, that is, each year's egg production is a constant percentage of the preceding year's production (88 per cent in the group of fowl studied). Since the exponential law is the same as the law of monomolecular change in chemistry, and since the course of egg production with age may be taken as an index of the course of senescence of organs, or tissues limiting egg production, it is suggested that this exponential law of egg production substantiates the idea that senescence is a physicochemical process the course of which is limited by a chemical reaction. It is shown that the exhaustion of the oocytes is not likely to be the factor limiting the course of egg production.

There is a good deal of published data on egg production with age up to 4 years. We are not, however, acquainted with statistically significant data after this age. This scarcity of data after 4 years is due to the difficulty of keeping records of egg production after 4 years of birds whose "normal average expectation of life *at birth* is not substantially

[3] Curtis, M. R., Quoted by Pearl in *Maine Agric. Exp. Station, Bull. 205*, 1912.
[4] Pearl, R., *Maine Agric. Exp. Station, Bull. 205*, 1912.

more than 2 years."[5] The relatively large amount of data up to 8 years presented in this communication was made possible by combining data obtained at other places with the data obtained at this station. We take much pleasure in expressing our indebtedness to J. H. Bardsley, of the New Mexico Station, J. E. Daugherty, of the California Station, B. F. Kaupp, of the North Carolina Station, J. H. Martin, of the Kentucky Station, H. G. May, of the Rhode Island Station, and L. H. Schwartz, of the Purdue Station, for their cooperation in obtaining the data summarized in Table I.

We have also included in our averages the data given by E. D. Ball, G. Turpin, and Byron Alder in the Utah Agricultural Experiment Station Bulletins.[6]

[5] Pearl, R., The biology of death, Monograph on Experimental Biology, Philadelphia and London, 1922, 63.

[6] Ball, E. D., Turpin, G., and Alder, B., *Utah Agric. Exp. Station, Bull. 135*, 1914. Ball, E. D., Alder, B., and Egbert, A. D., *Utah Agric. Exp. Station, Bull. 148*, 1916.

FURTHER EXPERIMENTS ON THE ABSORPTION OF IONS BY PLANTS, INCLUDING OBSERVATIONS ON THE EFFECT OF LIGHT.

By D. R. HOAGLAND and A. R. DAVIS.

(From the Division of Plant Nutrition, College of Agriculture, University of California, Berkeley.)

(Received for publication, July 16, 1923.)

Several investigations dealing with the penetration of ions into the cell sap of *Nitella*[1] have been reported recently by Osterhout,[2] Irwin,[3] Brooks,[4] and by the writers.[5] It has been proved by the experiments already described that most of the inorganic elements of the cell sap are present in dissociated form, and that these plant cells are able to cause the movement of ions from a solution of low concentration into one of higher concentration. As suggested in a previous article, this condition requires that energy relations be taken into account, in other words, the plant apparently must do work in absorbing ions from dilute solutions. If this is the case, it is reasonable to suppose that for autotropic plants, light, either directly or indirectly, is necessary to the processes of absorption, since light furnishes the ultimate source of energy to the plant. We have, therefore, made observations on the removal of certain ions from dilute solutions by cells of *Nitella clavata* kept under varying conditions of illumination. In the preliminary experiments, qualitative tests for the removal of chlorine from solution were made by comparisons of the turbidity developed when the chloride was precipitated by the

[1] As noted in a previous paper, the observations of Wodehouse on *Valonia* in 1917 first directed the attention of the writers to the advantage of supplementing their absorption studies on higher plants by the use of individual plant cells. At that time, Dr. Nathaniel Gardner kindly suggested the possibility of *Nitella*.

[2] Osterhout, W. J. V., *J. Gen. Physiol.*, 1921–22, iv, 275.

[3] Irwin, M., *J. Gen. Physiol.*, 1922–23, v, 223.

[4] Brooks, M. M., *J. Gen. Physiol.*, 1921–22, iv, 347.

[5] Hoagland, D. R., and Davis, A. R., *J. Gen. Physiol.*, 1922–23, v, 629.

addition of $AgNO_3$. These tests served to show the very marked influence of light on the absorption of chlorine but reliable quantitative measurements of this element could not be made by this method.[6] Other experiments were then carried out and entirely satisfactory results were obtained by using the Volhard method for the determination of chlorine. The general procedure was as follows:

Solutions were prepared of 0.01 M KH_2PO_4 plus 0.001 M or 0.0005 M KCl, and to definite amounts of these solutions (usually 200 or 600 cc.) were added equal weights of *Nitella*. The entire mass of cells used for each series of solutions was very thoroughly washed with tap, and then with distilled water. The cells were drained free of excess water and divided into equal portions, each mass of cells being quickly weighed to within approximately 0.1 gm. In most of the experiments 10 gm. of cells were added to 200 cc. of solution. The particular cells employed were quite small, as a rule, and practically no injured or dead cells were present as far as could be observed. In several of the experiments, the plants had previously been kept in peat water (which had a slightly acid reaction) and were free of surface deposits. In other experiments plants from tap water were used and these showed deposits of calcium salts on the surface. (These occur in the form of bands on the larger cells and at the tips of the very small cells.) After the cells were placed in contact with the solutions, the lipped beakers containing them were covered with watch-glasses, and those containers which were to be kept in the dark had inverted over them two-liter beakers coated with black asphalt paint. These large beakers were slightly raised at the bottom, so as to permit ready access of air. All containers were placed on a table in a well lighted room. There was a slight difference in temperature between the two sets of beakers in favor of those in the dark. However, this amounted to only a few tenths of a degree centigrade, and other experiments showed that this error was not responsible for the differences observed. The duration of

[6] Apparently soluble material from the *Nitella* tissue either prevented the complete precipitation of the chloride, or the colloidal state of the suspended chloride was altered. Determinations on the basis of turbidity always indicated a lesser amount of chloride than in the standard even when use of the Volhard method showed no absorption of Cl had taken place.

the period allowed for absorption was from 1 to 5 days. At the end of this period, the solutions were drained from the cells, made up to the original volumes, and analyzed by the method already indicated. In this way, determinations were made of the chlorine absorbed in full light and in the complete absence of light, and also with different exposures to light each day; *i.e.*, 1, 3, 5, and 8 hours. In addition, certain cells were kept in what was designated "dim" light. This was accomplished simply by wrapping the beaker with a piece of heavy white paper. In some cases, the watch-glass placed over the beaker was coated with black paint to dim the light still more. In all the experiments which are reported, the cells appeared to be as turgid and healthy at the end of the experiment as at the beginning. In a few cases, the cells kept in darkness or under the shortest exposure to light were injured, apparently because of bacterial growth, and these experiments were discarded.

The solutions were made up with KH_2PO_4, in order to maintain an acid reaction, since it was found that chlorine was absorbed more readily from an acid solution than from an alkaline one. The initial reaction of these solutions was approximately pH 5.0, but after contact with the plants for several days, the reaction changed to a higher pH, usually about 5.4 or 5.6. This change of reaction occurred even when cells were used which had no visible deposits of calcium salts on their surfaces, and was presumably brought about by a selective absorption of ions.

By referring to Table I, it will be seen that only very small amounts of chlorine were removed from solution when cells were kept in the dark and that increasing the number of hours of exposure to light also increased, in general, the absorption of chlorine. There was complete general consistency in all experiments, including some not reported. In several instances, all the chlorine was removed from solution, so that no test could be obtained with silver nitrate. Of course, no quantitative comparisons can be made between different experiments, since the light varied from day to day, according to weather conditions. To overcome this variable, it is planned in future work, to use controlled artificial illumination.

Several experiments of a similar character were carried out with KBr, and KI (Table II), and the same results were obtained as with

TABLE I.

The Effect of Light on Absorption of Cl by Nitella.

Solution employed: 0.01 KH_2PO_4 + KCl, as noted.

Condition of illumination.	Cl remaining in solution, P.P.M.			
	Experiment No.			
	1*	2*	3†	4*
	Original concentration of KCl.			
	0.0005 M (17.8 P.P.M. Cl)	0.001 M (35.5 P.P.M. Cl)	0.001 M (35.5 P.P.M. Cl)	0.001 M (35.5 P.P.M. Cl)
Full daylight	0	0	3	2
Dim light	1.5	14	35	27
Light 9 a.m. to 5 p.m.	5.0		16	10
" 11 " " 4 "	13.5	27	22	15
" 11 " " 2 "			26	25
" 11 " " 12 m.				28
Dark		31		34

* = Experiment continued 4 days
† = " " 5 "
200 cc. solution and 10 gm. *Nitella* used in all experiments.

TABLE II.

The Effect of Light on Absorption of Br by Nitella.

Solution employed: 0.01 KH_2PO_4 plus KCl, KBr, or KI, as noted.

Condition of illumination.	Br. remaining in solution, P.P.M.		I remaining in solution, P.P.M.	Cl remaining in solution, P.P.M.
	Experiment No.			
	1*	2†	3†	4†
	Original concentration.			
	80	74	111	34
Full daylight	2	9	86	4
Dim light	54	19	86	16
11 a.m. to 2 p.m.	34			
Dark	68	56	107	30

* = Experiment continued 4 days
† = " " 5 "
200 cc. solution and 10 gm. *Nitella* in all experiments.

KCl, except that the iodine was less readily absorbed than the chlorine or bromine.

The question then arose as to whether it could be shown directly that penetration of ions into the cell sap was affected by the illumination. It was not found convenient to test this point with chlorine since a large concentration of this element was already present in the cell sap. Br and NO_3, however, could be used for the purpose. In the first test with nitrate, the solution contained 0.01 M KH_2PO_4 plus 0.005 M KNO_3. After 8 days at room temperature, averaging 20°C., very heavy tests for NO_3 were found in the sap from all the cells kept in the light and only slight tests in the sap from the cells kept in the dark. Later, another experiment was made in which the beakers of solution (containing 0.01 M KNO_3 and 0.01 M KH_2PO_4) were both placed in a water bath at 28°C., one beaker being painted black and covered with a black watch-glass. After 3 days, samples of sap were obtained from the larger cells from both beakers, and an estimate of the nitrate made by the use of the phenoldisulfonic acid method. 106 parts per million NO_3 were present in the sap from the cells kept in the light and 56 parts per million in that from the cells kept in the dark. Under the conditions of immersion in the water bath, even the unpainted beaker received only a dim light, otherwise a greater difference might have been found.

These experiments were made to test for the penetration of Br. The solutions contained 0.01 M KH_2PO_4 (pH 5.0) and 0.01 M KBr. Two of the experiments were carried out at room temperature and the other at a temperature of 25°C., maintained approximately constant by the use of a water bath. The time of exposure was 4 or 5 days. This ion was estimated colorimetrically by the formation of a fuchsin dye which dissolves in chloroform.[7] The test is very delicate, but not adapted to give accurate quantitative data. However, the comparative values now under consideration were unquestionably significant. In each experiment, the concentration of Br in the sap obtained from cells kept in the light was approximately three times that of the sap from cells kept in the dark.

It would appear, therefore, that removal of ions from dilute solution and penetration into the cell sap are affected significantly by

[7] Denigès, G., and Chelle, L., *Ann. chim. anal.*, 1918, xxiii, 81.

the conditions of illumination. Other experiments with nitrate, it may be added, suggest that any considerable absorption from solution also is accompanied by penetration of the ion into the cell sap.

There can be only two explanations of the foregoing results; either light is important as a factor because it is a source of energy, or else permeability is directly influenced by light. The former explanation seems to us to be the more probable, when we take into consideration the relations which exist between the concentration of the cell sap and the culture medium. As bearing on this question, we may cite some data obtained by Dore[8] of this laboratory in the course of an investigation on the types of carbohydrates formed by *Nitella* and *Elodea*. In the former plant, he found only a trace of reducing sugar, even when the plants were removed from solution during the most active period of photosynthesis. It is conceivable that energy exchanges involved in absorption cannot proceed readily in the dark with the reserve of carbohydrate present. Irwin,[3] in a recent article, states that she has been able to follow the absorption of chlorine during the growth of *Nitella* cells. The general conclusion which we draw from these various experiments is that absorption of ions from dilute solutions is intimately related to processes of growth and metabolism involving energy exchanges.

The mechanism by which energy is utilized in the absorption of ions is not clearly understood at present. In *Nitella*, the normal functioning of the mechanism is easily disturbed. In this general connection, it may be of interest to mention several methods by which chlorine can be made to diffuse out of the cells, other than by the effect of an unfavorable culture solution. If a stream of air is passed through the solution containing a relatively high proportion of CO_2, chlorine will soon appear in the outside solution. Another method is to place the cells in a non-toxic solution of calcium and potassium nitrates and pass through the solution a direct electric current of very low intensity, for example, 25 milliamperes, for a period of from $\frac{1}{2}$ to 1 hour. If the cells are later subjected to normal conditions, this chlorine may be reabsorbed in the course of a few days.

[8] Dore, W. H., (unpublished data).

Chlorine may also be caused to diffuse out of a mass of healthy cells by lowering the temperature to 5°C. Subsequent reabsorption may occur here also after a few days at room temperature.

Effect of One Ion on Another at Low Concentration.

Osterhout[9] has proved that certain ions penetrate more rapidly into the cell from an unbalanced solution than from a balanced one. Antagonism between cations is especially pronounced. The observations have been made on solutions of relatively high concentration and the investigator of plant nutrition is interested also in the interrelations of ions where they exist in low concentrations, such as in ordinary soil solutions. In order to gain a further insight into these latter relations we have performed various experiments on *Nitella* and also on higher plants, especially barley. The latter experiments are being reported elsewhere, and will be referred to here only incidentally.

Two experiments were made on *Nitella* with balanced and unbalanced solutions at different concentrations. In the lower concentrations, approximately to 0.03 M no injury was produced by immersing the cells in KNO_3 solutions, even in the absence of calcium. This was shown both by the appearance of the cells and by the fact that no chlorine diffused out of them. A sharp break occurred with 0.06 M KNO_3 at which concentration the cells became definitely injured and very marked tests for chlorine were given by the solution within less than 48 hours.

The addition of 0.01 M $Ca(NO_3)_2$ completely overcame the toxic effect of KNO_3 at 0.01 M concentration. It may be remarked that the exact concentration at which injury will be produced will vary with different cells. When the cells are in a very early stage of growth, they will be injured by lower concentrations in either balanced or unbalanced solutions. In these stages, the total concentration of the cell sap is probably less than that of older cells.

The next studies to be made were planned to determine, if possible, whether one ion could influence the absorption of another ion from

[9] Osterhout, W. J. V., Injury, recovery, and death, in relation to conductivity and permeability, Philadelphia and London, 1922.

very dilute solutions, where toxicity was not a factor. Accordingly, observations were made on the penetration of NO_3 into the cell sap (Table III) and also on its removal from solution (Table IV), as

TABLE III.

Effect of Various Anions on Penetration of NO_3 into Vacuolar Sap of Nitella.

Solution.	Duration of experiment.	Diphenylamine test for NO_3.
Experiment 1.		
	days	
A. 0.01 M KH_2PO_4 plus 0.005 M KNO_3		Very heavy test in all cells.
B. 0.01 " " " 0.005 " " plus 0.005 M KBr	26	No test in any cells.
C. 0.01 M KH_2PO_4 plus 0.005 M KNO_3 plus 0.005 M KCl		" " " " "
Experiment 2.		
A. 0.03 M KH_2PO_4 plus 0.005 M KNO_3		Very heavy test in all cells.
B. 0.03 " " " 0.005 " " plus 0.005 M KCl		No test in any cells.
C. 0.03 M KH_2PO_4 plus 0.005 M KNO_3 plus 0.005 M KBr	15	" " " " "
D. 0.03 M KH_2PO_4 plus 0.005 M KNO_3 plus 0.005 M K_2SO_4		Very heavy test in all cells.
Experiment 3.		
A. 0.01 M KH_2PO_4 plus 0.001 M KNO_3	6	A. Heavy test in all cells.
B. 0.01 " " " 0.001 " " plus 0.001 M KCl		B. No test in any cells.
		C. Trace in most cells.
C. 0.01 M KH_2PO_4 plus 0.001 M KNO_3 plus 0.01 M K_2SO_4	11	A. Heavy test in all cells. B. No test in any cells. C. Heavy test in all cells.

influenced by the presence in the latter of Cl, Br, I, or SO_4 ions. Since chlorine is absorbed more readily from acid solutions, 0.01 M KH_2PO_4 (in one case 0.03 M) was used, which gave an initial reaction of approximately pH 5.0. KNO_3 was added in 0.001 M, 0.005 M,

TABLE IV.

Effects of Various Anions on Removal of NO_3 from Solution by Nitella.

Solution.	Duration of experiment.	NO_3 removed from solution, P.P.M.	Remarks.
Experiment 1.			
	days		
A. 0.01 M KH_2PO_4 plus 0.0005 M KNO_3		21	3,000 cc. solution and 75 gm. *Nitella*.
B. 0.01 " " " 0.0005 " " plus 0.0005 M KCl	8	8	
C. 0.01 " " " 0.0005 " " " 0.005 " "		5	
Experiment 2.			
A. 0.01 M KH_2PO_4 plus 0.0005 M KNO_3		At end of 5 days, NO_3 completely removed from all solutions except C and D, which contained 1.2 P.P.M. NO_3 and 9.3 P.P.M. NO_3, respectively.	400 cc. solution and 20 gm. *Nitella*.
B. 0.01 " " " 0.0005 " " plus 0.0001 M KCl			
C. 0.01 " " " 0.0005 " " " 0.0003 " "			
D. 0.01 " " " 0.0005 " " " 0.0005 " "	5		
E. 0.01 " " " 0.0005 " " " 0.0005 " K_2SO_4			
F. 0.01 " " " 0.0005 " " " 0.005 " "			
G. 0.01 " " " 0.0005 " " " 0.03 " "			
Experiment 3.			
A. 0.01 M KH_2PO_4 plus 0.0002 M KNO_3		A. No test outside solution	400 cc. solution and 20 gm. *Nitella*.
B. 0.01 " " " 0.0002 " " plus 0.0005 M KCl		B. Heavy test outside solution	
C. 0.01 " " " 0.0002 " " " 0.01 " K_2SO_4	2	C. Medium test outside solution	

and 0.01 M in concentration, respectively, in three different experiments. To certain of the nitrate solutions KCl, KBr, KI, or K_2SO_4 was added, several different concentrations being used (see Table III). After the cells had been in contact with these solutions for periods which varied from 6 to 26 days, about 50 of the larger cells (from $\frac{1}{2}$ to 1 inch in length) from each solution were tested for nitrate by breaking the cell and ejecting the drop of sap into a large drop of diphenylamine reagent. These tests showed very consistently that Cl, Br, and I ions, even in very low concentrations had a decided effect in decreasing the rate of penetration of NO_3, whereas SO_4 ions had a far smaller effect. In one instance, 0.01 M K_2SO_4 was less effective than 0.0005 M KCl.

We next decided to find out how the above mentioned ions would affect the removal of nitrate from solution, as shown by tests on the culture solutions themselves, rather than on the cells. The results of these experiments are shown in Table IV. They are entirely confirmatory of the qualitative tests made on the cell sap. Extremely small concentrations of chlorine ion were able to depress the rate of removal of NO_3 from solution, while SO_4 has a relatively slight effect. Since PO_4 ion was present in all solutions in at least 0.01 M concentration, evidently this ion also does not have a great influence on the absorption of nitrate. These facts do not mean, of course, that nitrate may not be removed from solution by actively growing or metabolizing cells, even in the presence of chlorine. Obviously this must be the case, and it can be shown experimentally. In some cells from solutions containing chloride, slight nitrate tests were given by the residue of the cell after breaking when the sap itself showed no test. Furthermore, NO_3 is subject to reduction in the living cell and can be slowly utilized to form other nitrogenous compounds. It may, perhaps, be suggested as a possibility that nitrate penetrates into the sap only after a certain concentration of the ion, or of some intermediate compound, is attained in the protoplasm.

It was shown in a previous communication[5] that NO_3 penetrated into the cell sap more readily from acid than from alkaline solutions. These observations have been confirmed, and the same relation can be demonstrated by determining the quantity of NO_3 removed from the culture medium (Table V). It also appears that the reac-

tion of the solution may have like influence on the removal of chlorine. Irwin[9] also suggests that such a relation exists between the absorption of anions and hydrogen ion concentration. With higher plants growing in culture solutions containing nitrate, it has been found by Theron,[10] and by one of the writers[11] that the equivalents of anions absorbed exceed those of cations at an acid reaction and that the opposite is true when absorption takes place from a solution of alkaline reaction.

TABLE V.

Removal of NO_3 from Acid and Alkaline Solutions by Nitella.

Solution.	Initial reaction pH.	Final reaction pH.	Duration of exposure.	Nitrate removed from solution, P.P.M.	Remarks.
Experiment 1.					
			days		
A. 0.02 M KH_2PO_4 plus 0.001 M KNO_3	5.0	5.5	6	22	400 cc. solution and 15 gm. *Nitella*.
B. 0.01 M " " NaOH plus 0.001 M KNO_3	8.4	7.8	6	11	
Experiment 2.					
				Beaker No. 1 2	
A. 0.01 M KH_2PO_4 plus 0.005 M KNO_3	5.0		7	20 17	400 cc. solution and 15 gm. *Nitella*.
B. 0.005 M " " NaOH plus 0.0005 M KNO_3	8.4		7	1 0	

The relation of cations to the removal of anions in dilute solution will require further experimentation, but the data at present available indicate that an anion cannot be absorbed readily when associated with a slowly absorbed cation. Thus with *Nitella*, chlorine was found to be absorbed far more rapidly from solutions containing K or Rb as the cation than from solutions containing only Ca or Mg (Table VI). A small proportion of Ca ion in addition to K ion pro-

[10] Theron, J. J., *Univ. Calif. Pub. Agric. Sc.*, 1923 (in press).

[11] Hoagland, D. R., *J. Agric. Research*, 1919–20, xviii, 73.

duced a relatively small effect at these low concentrations. We cannot be certain at present whether the effect is significant.

In experimenting with barley plants, we have found[12] that a slowly penetrating ion has a pronounced influence in depressing the rate of absorption of an ion of opposite charge. Thus potassium is absorbed much more rapidly from the chloride than from the sulfate, when solutions of 0.005 M concentration are employed. Investigations on the absorption of ions by barley plants also show that a relatively high concentration of one cation may depress the rate of absorption of another cation contained in the same solution. For

TABLE VI.

Effect of K, Ca, Mg, and Rb on Absorption of Cl by Nitella.

Solution.	Initial reaction pH.	Final reaction pH.	Duration of exposure.	Chlorine removed from solution, P.P.M.		Remarks.
				Beaker No. 1	2	
Experiment 1.						
			days			
A. 0.01 M $Ca(H_2PO_4)_2$ plus Ca $(OH)_2$ plus 0.001 M $CaCl_2$	5.0	5.4	14	5	2	400 cc. solution and 15 gm. *Nitella.*
B. 0.01 M KH_2PO_4 plus 0.002 KCl	5.0	5.6	14	42	45	
Experiment 2.						
A. 0.001 M KCl	5.6	7.3	14	26	24	200 cc. solution and 10 gm. *Nitella.*
B. 0.001 " RbCl	5.6	7.3	14	20	24	
C. 0.001 " $CaCl_2$	5.5	7.3	14	7	4	
D. 0.001 " $MgCl_2$	6.0	7.3	14	5	9	

example, sodium may decrease the rate at which calcium or potassium is removed from solution. These effects cannot be explained by differences in the amounts of water transpired. Preliminary tests with *Nitella* also indicated that sodium may depress the rate of absorption of potassium in solutions of low concentration, but the results are not sufficiently conclusive to warrant any assured statements.

One of the important factors to be considered in connection with the absorption of ions by plant cells is temperature. We have at-

[12] Hoagland, D. R., *Univ. Calif. Pub. Col. Agric. Techn. Papers*, 1923 (in press).

tempted some experiments along this line and while it is not intended to discuss temperature effects in this article, one or two of our observations may be of interest. The penetration of nitrate into the cell sap was tested at two temperatures held only very approximately at 12.5° and 22.5°C. After 13 days, the sap of the cells in the solution at 12.5°C. showed only a trace of nitrate, while that from the cells kept at 22.5°C. contained 220 parts per million according to analysis by the phenoldisulfonic method. Other experiments were undertaken to determine the rate of removal of NO_3 ions from solutions kept at 15°, 20°, 25°, and 30°C. The cells were contained in tall beakers which were immersed in the temperature baths. These latter were of dimensions just sufficient to accomodate the beakers and consequently the cells were illuminated very feebly. Under these circumstances, only small amounts of nitrate were removed from the 0.001 M solutions and temperature effects were not very significant. In a later experiment, the illumination of the cells was increased by suspending over each beaker a 100 Watt electric light. Larger amounts of nitrate were absorbed in this experiment, and four times as much was removed at 25° as at 15°C. It is apparent that such studies should be made under conditions of adequate illumination and new experiments are planned with this idea in mind.

DISCUSSION.

On the basis of the experiments referred to in this article, simple as they are, a number of important points are opened up for discussion. It seems justifiable to conclude, first, that even at very low concentrations, one ion may influence the absorption of another ion present in the same solution. This may not be the result of any change in the general permeability of the cell, such, for example, as would be shown by conductivity measurements. In fact, these relations may exist in a solution which presumably is capable of maintaining normal permeability. It is also obvious that with a given state of permeability, the different ions of the same solution may enter the cell at different rates. The interrelations of ions in absorption from dilute solutions extend not only to ions of like charge, but also to those of opposite charge.

Osterhout has suggested that effects of antagonism may be relatively unimportant in very dilute solutions. According to this investigator,[13] antagonism is defined as follows: "When toxic substances act as antidotes to each other this action is called antagonism." Under this definition, presumably, it would not be correct to describe as cases of antagonism those effects of one ion on the absorption of another from dilute solutions, such as we have described. From the standpoint of the nutrition and general metabolism of the plant, however, these latter phenomena are also of very great interest, because they relate to the mechanism by which essential elements are removed from culture media.

It is also essential to consider why different ions are absorbed from the same solution at such different rates. It seems to be a rather general observation that the sulfate ion is absorbed only very slowly by plant cells. For example, after 3 weeks, *Nitella* cells removed all of the chlorine from a solution of 0.0005 M concentration, but only a very slight proportion of the sulfate which was present in equivalent amount. This cannot be explained on the ground of slow utilization of the ion in the ordinary sense of the term, since chlorine ion is absorbed very readily, and yet it exists in the cell mainly, if not entirely, in uncombined state. The relative effects of chlorine and sulfate on the absorption of nitrate do not correspond to valence relations or to any simple chemical or physical properties of these ions. It might be suggested as an hypothesis that sulfate ions do not penetrate readily to those protoplasmic surfaces at which the chlorine exerts its influence on the absorption of nitrate, perhaps in connection with certain intermediate chemical compounds involved in the process of absorption. The relative rates of penetration of SO_4 and Cl into gels have not been found by Stiles[14] to show the same relations as in living cells. Loeb[15] shows that the influence of salts on the rate of diffusion of acids through collodion membranes is not the same as with membranes of living cells. In other words, in all these cases, the nature of the membrane as well as that of the ions, must be taken into account.

[13] Osterhout, W. J. V., *Science*, 1916, xliv, 318.
[14] Stiles, W., *Biochem. J.*, 1921, xv, 629.
[15] Loeb, J., *J. Gen. Physiol.*, 1922–23, v, 255.

It is not clear, as yet, to what extent the recent discoveries of Loeb with regard to ion protein relations will serve to explain the effect of hydrogen ion concentration on ion absorption. There is undoubtedly such an effect as our experiments have shown and as has been suggested by Irwin.[3] It does not always happen, however, than an anion is absorbed more readily than the cation from an acid solution. For example, potassium may be absorbed by barley plants more readily than sulfate, even at pH 5.0 to 3.2. It is not to be expected, however, that any simple explanation will suffice. As Osterhout[9] has pointed out, the cell undoubtedly forms a dynamic system in which "life is dependent upon a series of reactions which normally proceed at rates bearing a definite relation to each other."

Raber[16] has recently attempted to explain by one general theory the various types of antagonisms met with in studies on *Laminaria*. It would appear from the considerations advanced in the present paper, that an explanation of this type would not extend to the absorption of ions from very dilute solutions.

The goal of the present day plant physiologist is to explain the growth of plants in terms of the same laws as those used by the chemist and physicist. In a recent book, Lewis and Randall[17] express an opinion to the effect that truly quantitative understanding of chemical reactions can be attained only by the application of thermodynamic principles. It cannot be expected, therefore, that any completely quantitative view of the physiological processes of plants will be available until the free energy relations of the system are worked out, and this statement seems to apply to the absorption of ions, as well as to other processes.

SUMMARY.

1. The conditions of illumination were found to exert a very significant influence on absorption of ions from dilute solution by *Nitella*. These conditions were also found to influence the penetration of Br and NO_3 into the cell sap.

[16] Raber, O., *Bot. Gaz.*, 1923, lxxv, 298.

[17] Lewis, G. N., and Randall, M., Thermodynamics and the free energy of chemical substances, New York, 1923.

2. It is concluded that absorption of ions by plants from dilute solutions involves energy exchanges, with light as the ultimate source of the energy. It is suggested that the absorption is intimately related to growth and metabolism.

3. One ion may affect the removal from solution or penetration into the cell sap of another ion present in the same solution, even in solutions of extremely low concentration. It is probable that all three types of relations may exist—anion to anion, cation to cation, and anion to cation.

4. The sulfate and phosphate ions exerted far less influence on the absorption of nitrate than did chlorine and bromine ions. It is suggested as a possibility that sulfate does not penetrate readily to those surfaces at which chlorine, bromine, nitrate, and other ions may become effective.

CHANGES IN THE STABILITY AND POTENTIAL OF CELL SUSPENSIONS.

I. The Stability and Potential of Bacterium coli.

By ARNOLD H. EGGERTH.

(From the Department of Bacteriology, Hoagland Laboratory, Long Island College Hospital, Brooklyn.)

(Received for publication, June 4, 1923.)

In a previous investigation (Eggerth and Bellows, 1921–22) a strain of *Bacterium coli* was used that agglutinated in acid salt mixtures at pH 3.3, and remained unagglutinated at pH 3.0 and 3.5. It was observed that when agglutination at pH 3.3 was completed (after 1 or 2 hours at 40°C.), that if the tube was thoroughly shaken, the agglutinated bacteria went into even suspension and failed to flocculate again even when kept for days at that pH. Frequently microscopic examination showed that agglutination in small microscopic flocculi persisted, but if the treatment with acid was continued for another hour or two, vigorous agitation produced reemulsification so complete that not a trace of microscopic flocculation was demonstrable. Furthermore, such an acid-treated suspension, when sedimented on the centrifuge and resuspended in a series of buffer solutions, failed to agglutinate at any reaction between pH 7.0 and 2.4.

The following experiment was undertaken. An 18 hour agar culture of this strain of *Bacterium coli* (which will henceforth be referred to as the Goldberg strain) was washed four times in distilled water. Suspensions were made in lactic acid-sodium lactate buffers at pH 2.4, 2.7, 3.3, 3.8, and 4.4, and in acetic acid-sodium acetate buffers at pH 5.0 and 5.6. These were incubated at 35°C. for 3 hours. The buffer solutions were made up according to Beniasch (1912), except that the final salt concentration was made 0.01 N. After incubation, the suspensions were sedimented on the centrifuge, taken up in distilled water, and distributed in the lactate series of buffers.

As it was found that the density of the bacterial suspension made some difference in the results, the *coli* suspensions were compared with a standard suspension of staphylococci, and the turbidity in the agglutination tubes was made equivalent to that of a staphylococcus suspension of 2 billion per cc.

The results of this experiment are shown in Table I. Treatment at pH 3.3 and reactions acid to this prevented agglutination unless

TABLE I.

pH at which incubation for 3 hrs. was maintained.	Flocculation. Lactate buffers. pH								
	4.7	4.4	4.1	3.8	3.5	3.3	3.0	2.7	2.4
2.4	−	−	−	−	−	−	−	−	−
2.7	−	−	−	−	−	−	−	−	−
3.3	−	−	−	−	−	−	−	−	−
3.8	−	−	−	−	−	+	+	+	+
4.4	−	−	−	−	−	+	+	−	−
5.0	−	−	−	−	−	+	−	−	−
5.6	−	−	−	−	−	+	−	−	−
3.3*	−	+	−	−	−	−	−	−	−
3.3†	−	+	+	+	+	+	−	−	−
3.3‡	−	−	+	+	+	+	−	−	−
Control	−	−	−	−	−	+	−	−	−

The final concentration of sodium lactate was 0.01 N. Temperatures = 35°C.
* Washed three times in distilled water after neutralization of sediment.
† " " " " 1 per cent NaCl.
‡ " " " " 2 " " $NaHCO_3$.

the acid-treated bacteria were washed. At pH 3.8 and 4.4 the effect was to widen the zone of flocculation. Treatment with buffers at pH 5.0 and 5.6 gave agglutination as in the control. Attempts to reverse the effect of acid treatment of these bacteria by neutralizing the sediment and washing with distilled water, 1 per cent NaCl, or 2 per cent $NaHCO_3$, changed the stability of the bacteria once more; flocculation reappeared, but the zone of flocculation was widely displaced toward the alkaline side.

Experiments were then undertaken to determine if any changes in the potential difference between the bacteria and the fluid accompanied these alterations in stability. For this purpose, a microcataphoresis apparatus made according to Northrop (1921–22) was used. The depth of the cell was 1 mm., and the fall in potential of the current was 5 volts per cm. The principles of this apparatus are fully explained by Northrop. In one way only was his procedure departed from: instead of making measurements at four depths in the upper half of the cell only, measurements were made in the middle of each sixth of the entire cell depth. Theoretically, the upper and lower halves of the Northrop cell are symmetrical; when using a new, clean cell, this is actually the case. But after a little use, the glass walls of the cell become coated with bacteria, as was described by Northrop (1921-22), and the migration of water along the cell wall is changed. As the bottom of the cell acquires a thicker coating of cells in a shorter time, the two halves are no longer symmetrical. In most of the observations for this paper, the rate of movement was distinctly slower in the lower half of the cell than in the upper, although this varied with the suspension used. In cataphoresing bacteria, the error produced in making measurements in the upper half of the cell only is not large; but when heavier cells, such as erythrocytes, are used, this error becomes very great.

The rate of movement of the bacteria is given in the figures as micra per second with a calculated fall in potential of 1 volt per cm. The potential in millivolts between the surface of the bacteria and the fluid is given as calculated from the observed velocity by means of the Helmholtz-Lamb equation, as discussed by Northrop and Cullen (1921–22). In these cataphoresis experiments, the bacteria were always washed four times in distilled water and cataphoresed on the same day. Unless otherwise specified, the buffer solutions were lactic acid-sodium lactate mixtures containing 0.01 N salt.

Different suspensions of *Bacterium coli* do not always have the same charge at a given pH. Thus, at pH 4.7, the cataphoretic potential of the Goldberg strain varies between 34 and 42 millivolts in the buffer used. Northrop and De Kruif (1921–22, *a*) noted similar variations in their suspensions of *Bacterium typhosum*, which they ascribed to differences in the age and condition of the suspen-

sion. No such variations, however, occurred when observations were made on the same preparation of *Bacterium coli* at the beginning and end of an experiment.

Fig. 1 shows the changes in charge of the Goldberg strain when incubated at 35°C. at different reactions. At pH 4.1 and 5.3 there was no appreciable change within 7 hours. At pH 3.8, the curve remained flat for 2 hours after which the negative charge diminished until at the end of 6 hours it was one-third of its original value. At more acid reactions, the velocity of this change in charge is greatly accelerated; at pH 2.4, the charge fell to zero within 45 minutes.

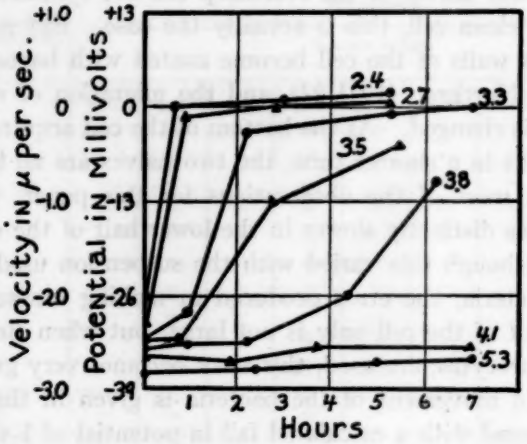

FIG. 1. Effect on the potential of *Bacterium coli* suspensions when incubated at 35°C. in 0.01 N lactate solutions at the pH indicated on the figure.

Similar experiments conducted with acetate and phthalate buffers, and with mixtures of HCl and KCl, gave the same results.

At intervals during this experiment, the *coli* suspensions were plated out on agar media to test for viability. Though microscopic agglutination interferes with the strict accuracy of the results, it seems that there is little cell death during the period of the flat portions of the curves, but that the bacteria are dying rapidly as the curves rise steeply. It is probable that cell death and change in charge are both results of profound physical and chemical alterations of the cell.

In Fig. 2, the charge of acid-treated bacteria is compared with that of normal controls at different reactions. Curve *A* gives the charge of acid-treated bacteria which were centrifugated and resuspended in the different buffer solutions without further washing. Curve *B* shows the same acid-treated bacteria, neutralized, incubated for 6 hours in a 0.01 N phosphate solution at pH 7.6, and then washed in distilled water. Acid treatment diminished the negative charge at all reactions between pH 6.4 and 2.4, especially at the acid end of this series. The attempt to reverse the effect of acid treatment (Curve *B*) produced only inconsiderable changes, most noteworthy being the increase of the positive charge at pH 2.7 and 2.4, which occurred very constantly in different experiments. When the acid-treated bacteria were washed three times in 1 per cent NaCl or 2 per cent $NaHCO_3$, curves were obtained that differed very little from Curve *B*, and are therefore omitted.

The exact position and inclination of Curves *A* and *B* of Fig. 2 varied somewhat in the different experiments as the initial charge and the duration of treatment varied. Prolonged acid treatment caused Curve *A* to rise much more steeply, and *vice versa*. Curve *B* also varied with the length of acid treatment and the length of the after treatment, lying sometimes to the left, sometimes to the right of Curve *A*, though never far from it.

Figs. 1 and 2 have to deal with the Goldberg strain of *Bacterium coli*. Ten other strains of this organism were isolated and tested for similar behavior. By referring to Table II it will be seen that each of the other ten strains has its original negative charge diminished by acid treatment, some even acquiring a considerable positive charge. The phenomenon here studied is not, therefore, a peculiarity of this one strain. No other species of bacteria have as yet been studied in this manner, but experiments with human and sheep erythrocytes show that similar changes of potential take place in red cells. These will be reported in a later paper.

When fresh washed bacteria (the Goldberg strain) were added to a suspension that had been incubated for some time at pH 3.3, it was found that all of the bacteria in the field, both new and old, moved with the same velocity (usually zero) when cataphoresed. When a suspension that had been treated at this reaction until the

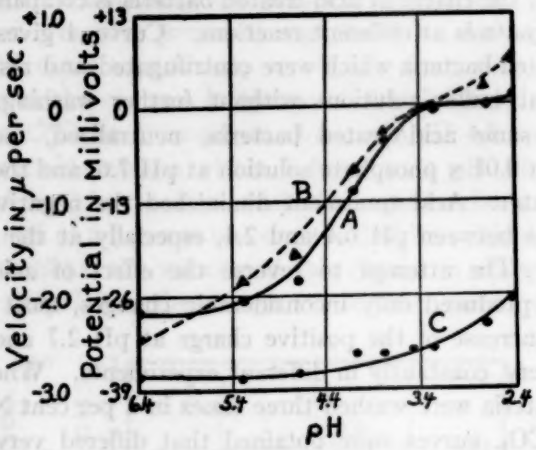

FIG. 2. The potential of *Bacterium coli* suspensions in 0.01 N lactate solutions; (*A*) after being incubated for 3 hours at 35°C. at pH 3.3, (*B*) after being thus treated at pH 3.3, then incubated for 6 hours at pH 7.6 and washed once in distilled water, and (*C*) the potential of washed but untreated *Bacterium coli*.

TABLE II.

Strain of *Bacterium coli*.	Fresh bacteria in fresh buffer. (1)	Fresh bacteria in acid, supernatant. (2)	Acid-treated bacteria in fresh buffer. (3)
	millivolts	*millivolts*	*millivolts*
Resa	−10.5	0	+3.0
Tucker	−16.0	0	+11.0
Siegel	−15.0	−7.5	+5.5
Wilkins	−22.0	−9.0	−5.5
Peck	−1.5	0	0
Matthews	−23.5	−5.5	−4.5
Penny	−16.0	+8.5	+7.5
Gert	−21.5	−20.0	−10.0
Mouse	−5.5	0	0
Cameron	−21.0	0	−2.5
Goldberg	−34.0	0	0

Potentials in 0.01 N lactate buffer at pH 3.0.

potential of the cells was zero, was centrifugated, and fresh cells added to the clear supernatant fluid, cataphoresis showed that the charge of the new cells was now zero, whereas fresh cells in fresh buffer at this pH usually have a charge of about 35 millivolts. Not only are the cells altered by acid treatment, but the fluid in which they are suspended acquires new properties.

In order to study this phenomenon in greater detail, an "acid extract" was prepared by adding a HCl-KCl mixture at pH 1.8 to a heavy suspension of *Bacterium coli* Goldberg, making a final salt concentration of 0.01 N. Enough alkali was liberated by the bacteria to depress the H ion concentration to a pH of 3.0 (colorimetric). After 4 hours at 35°C., the suspension was sedimented on the centrifuge and the clear supernatant fluid brought to pH 7.0. This neutralized "acid extract" gave a distinct turbidity with alcohol and foamed strongly on shaking; a micro Kjeldahl determination gave a total N of 10 mg. per 100 cc. Assuming this to be all protein nitrogen, the protein concentration was 1 part in 1,600.

The neutralized "acid extract" prepared in this way was now added in varying quantities to fresh cells in fresh buffer solutions, determinations being made immediately. Results are given in Fig. 3, which shows that even small amounts of the "acid extract" depress the charge to some extent, while larger amounts depress it a great deal especially in the more acid reactions.

When Fig. 3 is compared with Figs. 1, 2, and 3 of the paper of Northrop and De Kruif (1921–22, *b*), it will be seen that this "acid extract" behaves like any other protein in diminishing the negative charge on the bacteria. The "acid extract" is liberated from the bacteria, and then, in part, combines with their surfaces. It is possible that this protein is similar to that extracted by Arkwright (1914) from *Bacterium typhosum*, though the "acid extract" here described differs from Arkwright's extract in two important ways: it is not obtained at neutral reactions, and it does not flocculate at any pH. Undoubtedly Arkwright's extract contained a great deal of peptone and other substances from the media on which the germs were grown.

The other ten strains of *Bacterium coli* were now tested to see if they too produced an active "acid extract." The washed bacteria were treated at pH 3.0 for 10 hours at 35°C. (0.01 N lactate buffer)

and sedimented on the centrifuge. Fresh washed bacteria of the homologous strain were now emulsified in the clear supernatant fluids and cataphoresed (Column 2, Table 2). The sediments were suspended in fresh buffer at pH 3.0 and also cataphoresed (Column 3). In Column 1 are found the potentials of fresh washed bacteria at this pH. Although the results obtained are very diverse, they agree essentially with the facts ascertained by the more detailed study of the Goldberg strain.

We are now in a position to make a partial interpretation of the agglutination experiment shown in Table I. The bacteria treated at pH 3.3 and more acid reactions have become covered with a film of

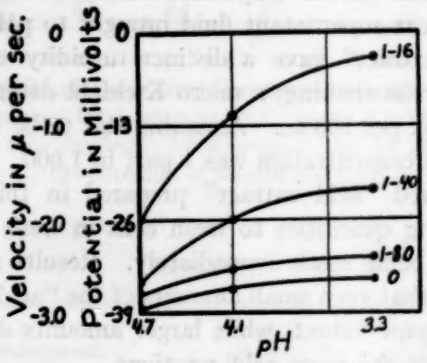

FIG. 3. The potential of washed but untreated *Bacterium coli* in 0.01 N lactate solutions to which "acid extract" has been added in a concentration indicated on the curves.

substance, probably a protein, derived from the bacteria themselves; this serves to stabilize the cell suspension, even when the cataphoretic potential is zero. Since Loeb (1922–23) has shown that true protective colloids, such as gelatin, maintain the stability of suspensions irrespective of the cataphoretic potential difference (by virtue of their higher attraction for the molecules of the solvent than for each other), it is probable that this protein acts as a protective colloid. When sedimented and suspended in fresh solutions, there is still enough of this protein adhering to the bacteria to act as a protective colloid and prevent agglutination. When this substance is removed by washing, the bacteria are now found to agglutinate at

more alkaline reactions than did the untreated bacteria. It is necessary to point out that bacteria that have been acid treated for a few hours, washed, and then placed in buffer solutions, may undergo still further changes in potential; while this fact has been verified in a preliminary way, it has not been studied in detail.

SUMMARY.

1. Stability and potential of *Bacterium coli* suspensions depend, not only on the strain of the organism and the medium in which it is suspended, but also on the previous treatment of the suspension, and the length of time it has been in the medium.

2. When treated at acid reactions, the negative charge on the bacteria is diminished; with some strains, a positive charge is acquired. Changes in stability accompany the changes in potential.

3. Washing acid-treated bacteria at neutral or slightly alkaline reactions does not restore the original potential; the zone of flocculation is moved toward the alkaline side.

4. These changes are due to two factors: the extraction of a soluble protein which combines with the surfaces of the cells, and a further irreversible change of the cell or its membrane.

BIBLIOGRAPHY.

Arkwright, J. A., *Z. Immunitätsforsch., Orig.*, 1914, xxii, 396.
Beniasch, M., *Z. Immunitätsforsch., Orig.*, 1912, xii, 268.
Eggerth, A. H., and Bellows, M., *J. Gen. Physiol.*, 1921–22, iv, 669.
Loeb, J., *J. Gen. Physiol.*, 1922–23, v, 479.
Northrop, J. H., *J. Gen. Physiol.*, 1921–22, iv, 629.
Northrop, J. H., and Cullen, G. E., *J. Gen. Physiol.*, 1921–22, iv, 635.
Northrop, J. H., and De Kruif, P. H., *J. Gen. Physiol.*, 1921–22, *a*, iv, 639.
Northrop, J. H., and De Kruif, P. H., *J. Gen. Physiol.*, 1921–22, *b*, iv, 655.

THE FUNCTION OF THE BRAIN IN LOCOMOTION OF THE POLYCLAD WORM, YUNGIA AURANTIACA.

By A. R. MOORE.

(*From the Zoological Station, Naples, Italy.*)

(Received for publication, June 9, 1923.)

As a result of his experiments on the polyclad worm, *Thysanozoon brocchii*, Loeb[1] concluded that spontaneous progressive movements were a function of the part of the body containing the brain. He suggests, however, that failure of spontaneous movement in decapitated *Thysanozoon* may be the result of chemical or physical constitution and that an alteration in the composition of the surrounding solution may cause the posterior half to show spontaneous movements.

The marine polyclad worm, *Yungia aurantiaca*, is favorable material for such studies and behaves similarly to *Thysanozoon* in that decapitated individuals no longer show progressive movements in sea water. Normal specimens of *Yungia* either creep about over surfaces by means of cilia and muscular contractions, or swim through the water using the two lateral halves of the body as swimming organs. Only forward locomotion is possible in this form. In starting to swim the animal bends the head and sides of the body ventrally. This is followed by an upward bending of the head and rapidly succeeding waves of dorsal and ventral flexion of the lateral halves, such waves beginning at the head on both sides and passing simultaneously to the posterior end. These movements exert a propeller-like action which drives the animal forward through the water.

The existence of two associated muscle groups which perform the swimming movements may be proven by selective stimulation of the respective motor elements. When either entire animals or decapitated pieces are put into strychnine sulfate solution, 1:100,000 in sea water,

[1] Loeb, J., Comparative physiology of the brain and comparative psychology, New York, 1900, 78, 79.

ventral flexion of the two lateral halves ensues, followed by rhythmically recurring contractions resembling the beating of a heart. Likewise, the dorsal musculature can be demonstrated by immersing the worms in nicotine solution 1:10,000 in sea water. In this case after 3 or 4 minutes the lateral halves bend dorsally, and decapitated pieces make spontaneous swimming movements.

The action of strychnine and of nicotine prove, therefore, that the body of *Yungia* apart from the head contains the locomotor apparatus necessary for progressive movement. It should be possible, then, to produce by means of proper stimulation progressive movement and swimming in decapitated pieces. This can in fact be accomplished in the following ways.

(1) By mechanical stimulation of the anterior median locus of decapitated pieces. Regular swimming movements of the sides occur as long as stimulation lasts. (2) By chemical stimulation of the motor elements. Phenol 1:25,000 in sea water causes decapitated specimens to swim through the water, the movements differing in no way from those of a normal worm except that they are slower. (3) By altering the ion ratio. Since all the locomotor elements requisite for spontaneous movements and swimming are present in decapitated pieces, it seems apparent that the failure of such pieces to make movements may be due to low irritability of the system. It was, therefore, attempted by the use of proper salts to bring about spontaneous movement in decapitated pieces. The experiments were successful in that spontaneous movements occurred when the pieces were put into solutions having an excess of Na and K ions over Ca and Mg. Actual swimming through the water did not take place, presumably owing to the injurious action of unbalanced salt solutions upon the tissues.

When the pieces of worm are put into pure NaCl or KCl solutions isosmotic with sea water (0.6 M) at once violent swimming movements begin and continue until the onset of disintegration. The latter happens in from 10 to 15 minutes. Both disintegration and swimming movements are inhibited by Ca and Mg ions in proper proportion. The following tables illustrate this point. The numbers indicate in each case the cubic centimeters of solution used.

NaCl.....	99	98	97	96	95
$CaCl_2$.....	1	2	3	4	5
Result.....	Movement.	Movement.	No movement.	No movement.	No movement.

NaCl.....	99	98	97	96	95
$CaCl_2$.....	1	2	3	4	5
KCl.....	1	2	3	4	5
Result.....	Movement in each solution.				

The action of Mg in antagonizing Na ions is shown by the following:

NaCl.....	98	96	92	84	76
$MgCl_2$.....	2	4	8	16	24
Result.....	Movement.	Movement.	Movement.	Slight movement.	No movement.

It thus requires about 30 molecules of $MgCl_2$ to inhibit the action of 100 $NaCl_2$ while only 3 molecules of $CaCl_2$ accomplish the same result. Consequently $CaCl_2$ is about ten times as powerful as $MgCl_2$ as an antagonist to NaCl. In a van't Hoff solution of the composition 100 NaCl + 2.2 KCl + 2 $CaCl_2$ + 12 $MgCl_2$ and in a solution composed of 100 NaCl + 2.2 KCl + 2 $CaCl_2$, the decapitated worms are entirely quiescent.

CONCLUSION.

Coordinated swimming movements in *Yungia* are not dependent upon the presence of the brain. The neuromuscular mechanism necessary for spontaneous movement and swimming is complete in the body of the animal apart from the brain. Normally this mechanism is set in motion by sensory stimulation arriving by way of the brain. The latter is a region of low threshold and acts as an amplifier by sending the impulses into a great number of channels. When the head is cut off these connections with the sensorium are broken, consequently peripheral stimulation does not have its usual effect. If, however, the motor nerves are stimulated directly as by mechanical stimulation of the median anterior region, then swimming movements

result. Also if the threshold of the entire nervous mechanism is lowered by phenol or by an increase in the ion ratios $\frac{\text{Conc. Na}}{\text{Conc. Ca}}$ and $\frac{\text{Conc. Na} + \text{K}}{\text{Conc. Ca} + \text{Mg}}$ then again peripheral stimulation throws the neuromuscular mechanism into activity and swimming movements result.

STUDIES ON TOXICITY.

By ELLIS I. FULMER and R. E. BUCHANAN.

(*From the Laboratories of Chemistry and of Bacteriology, Iowa State College, Ames.*)

(Received for publication, July 19, 1923.)

I. Criteria of Toxicity toward Microorganisms.

Extensive studies of the effect of toxic agents have been made in recent years upon both unicellular organisms and multicellular tissues. There have been many criteria of injury and death suggested and used, but terms have not always been employed by various writers in the same sense. The present paper is an attempt to indicate those criteria which have been found helpful when dealing with microorganisms, and particularly to point out the usefulness of staining reactions of cells in securing quantitative results on studies of toxicities.

In general any agency or material which produces an injurious effect upon the normal functions of a cell may be characterized as toxic. If two or more such substances produce equal harmful effects in equal lengths of time under otherwise uniform conditions they may be termed isotoxic. Commonly in such studies the observations are continued until all of the cells have undergone the maximum injury detectable by the criterion used.

Several methods of detecting cell injury on the one hand or toxicity of materials on the other have been proposed. Among those which are apparently most useful for studies of microorganisms in that results may be followed quantitatively are the following: changes in rapidity of multiplication or power of reproduction, changes in the electrical conductivity of the cells, changes in the permeability of the cell membranes, plasmolysis, variations in rate of metabolism, and changes in the ability of the cell to stain with various dyes.

In work with microorganisms, particularly with bacteria, the criterion of injury most frequently used has been the death of the cell as indicated by the complete loss of reproductive power. Antiseptics,

germicides, and disinfectants are toxic substances which more or less promptly kill cells as evidenced by their loss of reproductive capacity when replaced in a favorable environment. For representing graphically the relationships between time and the death of cells three types of graphs are in common use. The number or percentage of cells still living after varying intervals of contact with the toxic agent may be plotted against time, the curve thus secured is commonly termed the survivor's curve. Or the number of dead cells may be plotted against time of exposure, the resultant curve being termed the death curve. Or the number of cells which succumb during each equal interval of time may be plotted against time to constitute a rate curve, or distribution curve. The area under the rate curve is proportional to the total number of cells, the area to the left of any point on the x axis is proportional to the total number of cells dead, the area to the right is proportional to the total number of cells still living. It is possible, therefore, to derive each curve from either of the others. Much study has been devoted by microbiologists to the forms which these curves assume, and to the development of simple equations to represent them.

It is apparent that curves of the same general types as those used for survival or death of microorganisms may be used for the illustration of effects of toxic agents when some criterion of injury other than failure to reproduce is used. A curve of the type of a survivor's curve may be plotted to show the relationship between time and cells not exhibiting the particular effect of the toxic agent. Possibly the term survivor's curve may well be used for all curves of this type provided it is clearly understood in each case that the criterion of a "survivor" is not necessarily ability to reproduce. A rate or distribution curve may also be plotted. There is some danger of confusion, however, if the term death curve is applied to a plot of time against the number of cells showing the change to be observed regardless of the criterion used. Possibly such a curve may be termed a toxicity curve.

Many quantitative studies have been made of the effect of toxic agents in which changes in the electrical conductivity of cells has been the criterion of injury. The studies of Osterhout (1922), of Shearer (1919), and of Brooks (1923), are particularly interesting in their quantitative approach. The graph of electrical conductivity and

time may properly be developed as a toxicity curve, and solutions producing equal changes in electrical conductivity (or more usually the maximum change in conductivity) under otherwise identical conditions in equal lengths of time may be termed isotoxic with reference to this particular criterion. A similar toxicity curve might be constructed as a graph of permeability against time.

Of particular interest for the securing of quantitative data on toxicity are the methods which have been set forth in a series of papers from the University of Toronto on the general subject of chemical potential and toxicity. The detailed technique may be found in the papers by Miller (1920), Burgess (1920), Fraser (1920, 1921) and Fulmer (1921). In this series is sought an explanation of such phenomena as the increase of toxicity of phenol in aqueous solution when sodium chloride, alcohol or other substances are added. It was found that the addition of NaCl or alcohol to a solution of phenol in contact with an immiscible solvent such as toluene or kerosene causes the phenol to become less concentrated in the water and pass in part to the other solvent. This fact may be expressed in one of several ways: the chemical potential of the phenol is increased; the fugacity or tendency of the phenol to escape is increased; the solubility of phenol in the aqueous phase is decreased; or the phenol is driven into the non-aqueous phase. For these studies yeast cells were used generally, the criterion of injury first employed was the loss of the power of the cell to reproduce; *i.e.*, death rates were determined by plating. Later the acquisition of the ability of the cells to stain with an aqueous solution of methylene blue was taken as a second criterion of toxic action.

It would seem that this criterion of injury is worthy of more attention in toxicity studies. It has been shown that all cells which take up the stain are dead, that is, they no longer can reproduce. However, it was found that the ability to reproduce was lost *before* the cell acquired the ability to stain. In other words, cells which stain are dead, but a dead cell does not of necessity immediately acquire the ability to stain. The two phenomena are closely related, so that cell staining is a satisfactory criterion of toxicity.

Data are so much more rapidly obtained by observations on the staining reactions of yeast cells than by other methods of securing quantitative results on toxicity that it has been thought advisable to

secure and plot the data secured by this means. It is believed that results of value may be secured by a study of the shapes of survivor's and rate curves obtained.

Summary.—The acquisition of the ability of yeast cells to stain with methylene blue as an index to the toxicity of the solution in which the cells are placed is an important addition to the criteria which have been suggested for the detection of toxicity, and may be adapted to a wide variety of such studies.

Solutions which are isotoxic when one criterion of toxicity is used may not be isotoxic when some other criterion is used.

It is desirable that comparative studies should be made of the results secured by the use of various criteria of toxicity that have been suggested.

II. On Conformity of Yeast Survivor Curves to the Monomolecular Reaction Law.

Differences in opinion as to the interpretation to be attached to the form of the curve secured in the investigation of death rates of microorganisms by plotting the number of surviving organisms against time has led to two groups among students of the problem. The one group holds that experimental data thus plotted will give a logarithmic curve, and attempts to explain this on the basis of the laws of physical chemistry, even suggesting in some cases that the form of this curve is evidence of the monomolecular nature of the reaction. The second group holds that any resemblance between the curves such as has been described is superficial and fortuitous and that we must look to variations in resistance of individual cells as well as to the distribution of such variations to account for the form assumed by the curve.

Paul and Krönig (1897) were apparently the first to develop a satisfactory method of determining death rates of microorganisms and used it to secure data on the numbers of bacteria surviving after varying intervals of time in the presence of disinfectants. They showed that disinfection, that is, killing of the organisms present, did not occur at the same instant, but they did not deduce any exact relationships between number of survivors and time. Ikeda (1897) attempted to do this, and concluded that the ratio between the products of time and survivors was a constant. Renewed interest was taken in the problem when Madsen

and Nyman (1907) plotted data which showed excellent agreement between the curves secured and the curve of a monomolecular reaction. Chick (1908) repeated and extended these experiments and concluded: "An ideal case of disinfection, such as that of anthrax spores, may therefore be supposed, with experimental support, to proceed in accordance with the equation— $\frac{dn}{dt} = Kn$, an equation exactly similar in form to that expressing the course of a unimolecular reaction, or reaction of the first order." However, she noted discrepant results when a non-sporing organism (*Bact. paratyphosum*) was used, "the reaction velocity diminishing during disinfection more rapidly than is accounted for by the fall in number of the surviving bacteria." "This divergence is due to differences in resistance between individuals of the various ages contained in such cultures." Phelps (1911) on theoretical grounds, using the evidence presented by earlier writers, states: "The rate of dying, whether under the influence of heat, cold, or chemical poison, is unfailingly found to follow the logarithmic curve of the velocity law, if the temperature be constant. This curve never reaches a zero value, although approaching it indefinitely." Lee and Gilbert (1918) clearly differentiate the two schools of thought with reference to the disinfection process under the terms "vitalistic" and "mechanistic." The vitalistic concept is that "the progressive nature of the disinfection process is accounted for as being due to a type of biological variation, namely, *permanent differences* in the degree of resistance possessed by the various individual microorganisms of a 'pure culture.' This is commonly known as the theory of variable permanent resistance. The mechanistic conception of the process of disinfection is that it is an orderly time-process presenting a close analogy to a chemical reaction, the micro-organism and the disinfectant being regarded as the respective agents." They conclude as a result of data which they present: "The explanation why disinfection is not sudden, but gradual, and proceeds in close agreement with the well defined logarithmic law, known as the Mass Law, is best found in those chemical processes which proceed as 'uni-molecular' reactions." Recently Cohen (1922) has contributed a careful study of the effect of temperature and hydrogen ion concentration upon the viability of *Bact. coli* and *Bact. typhosum* in water. He concludes that with the exception of a more or less prolonged "induction period," "the mortality of bacteria whether by strong disinfectants or by milder agents follows the laws of logarithmic decline. It is shown that the course of the disinfection process can be expressed by mathematical relations comparable to those used in dealing with monomolecular chemical reactions." He also emphasizes that "The chance distribution of variable resistances assumed for bacteria is paralleled by the chance distribution of variable energy quotas in the molecules of a substance. Furthermore, the logarithmic rate observed, whether in chemical phenomena or in disinfection, is actually a statistical resultant of like significance in both."

During the development of the mechanistic theory, its opponents have not been idle. Reichel (1909) insisted that the results secured by Madsen and Nyman must

arise either from unequal cell resistance or from an unequal distribution of the organisms in the liquid. Loeb and Northrop (1917) comment on Chick's assumption that "'bacteria are killed by disinfectants at a rate corresponding to that of a monomolecular chemical reaction; *i.e.*, that in each interval of time the same percentage of individuals alive at this time is killed.' She was probably led to such an assumption by the fact that the ascending branch of the mortality curve in her experiments was generally very steep. The agencies used by her for killing the bacteria were so powerful that the ascending branch became almost a vertical line, thus escaping detection. Hence she noticed usually only the less steep descending branch which could be interpreted as a monomolecular curve for the reason that her experiments lasted only a short time." For the fruit flies with which they worked they conclude: "The fact that the frequency curve of deaths is that of a probability curve shows that the difference in the duration of life of different individuals for the same temperature is due to individual variation." Brooks (1919) after a review of the data concludes: "the course of such processes as hemolysis is very largely dependent upon variations in resistance among the different individuals, and secondarily upon the course of the fundamental reaction," and further "Unnatural assumptions would be requisite for the explanation of a resemblance between the course of such processes in general and that of a monomolecular reaction.

"The supposition that such a general resemblance exists is not supported by the available evidence." Peters (1920) studied the effect of mercury upon *Colpidium*, and concluded that death occurs in "such a way that there is a straight line relationship between the time and the logarithm of the percentage of survivors for a large part of the death process. The apparent logarithmic law can be interpreted in terms of the variation in the resistance of individuals of the protozoon culture to the toxic agent." Smith (1921) studied the survivor curve of *Botrytis* spores treated with phenol. He definitely shows the characteristic curve for these to be sigmoid in many cases, but as the quantity of phenol is increased there is a tendency for the maximum death rate to occur proportionately earlier and the curve approaches more nearly the logarithmic type. He states: "Both types of curves are shown to be explicable on the assumption that the individual spores differ in resistance and that a frequency curve showing the distribution in the resistance grades approaches the normal curve." Recently Reichenbach (1922) has reviewed the theoretical basis of disinfection. He concludes that it is quite impossible to imagine how two bacteria having equal resistances should die after different periods of time when subjected to the same unfavorable environment. While in many cases the survivor's curve is approximately logarithmic, this cannot be taken for granted under any given set of conditions until an actual test has been made.

It is apparent that it is a matter of unusual interest both from a theoretical and a practical point of view to determine whether or not

there is such substantial conformity to the logarithmic law as would permit of its utilization for estimation and comparison of the value of disinfectants.

Experimental.

It was pointed out by Fulmer (1921) that yeast cells taken from a wort culture in which the alcohol has reached a certain concentration, are much more resistant to phenol than cells from a culture which contains little alcohol. This fact was taken advantage of by using cells from a young culture for weak poisons and old cells with their greater resistance for the stronger poisons. Clark (1922) made a further study of the effect of the accumulation of alcohol upon the yeast.

The yeast was filtered, washed, and then suspended in the poison in the "rocker" tube at 25°C. At intervals a 1 cc. sample was placed in 1 cc. of 0.01 per cent methylene blue. The percentage of the cells stained by the dye was determined microscopically.

Survivor's curves were plotted using as an index of toxicity the per cent of cells stainable by methylene blue after a given time in contact with the poison. Twenty-seven curves were chosen at random for analysis in this paper. The tabular data are too voluminous to publish in this connection.

In each case the numbers of surviving organisms have been calculated in percentages. To make the various curves comparable the lengths of the abscissæ used to represent complete disinfection have been made equal, that is equal distances for the x axis represent equal percentages of the total disinfection time. A frequency curve showing the distribution of resistances was then plotted. Resistance is here interpreted as the time required under the conditions of the experiment for a cell to acquire stainability with the methylene blue. The logarithms of the numbers representing the survivors were also plotted.

A study of the twenty-seven groups of data thus plotted shows decided differences in the distribution of resistances. These are illustrated by solid blocks in the accompanying figures. It was found that the shape of the frequency curves secured by joining the tops of these blocks varied widely. The types of these curves may be included under several classes and subclasses. These groups together with the number of cases belonging to each are listed below.

I. No well marked maximum, distribution of resistances relatively uniform..... 4 cases.
II. With one maximum well marked..... 19 cases.
1. With maximum to the left of median..... 14 cases.
1st place, *i.e.*, the largest percentage of cells died in the first tenth of the time..... 9 cases.
2nd place..... 3 cases.
3rd place..... 2 cases.
2. With maximum median..... 2 cases.
3. With maximum to the right of median..... 3 cases.
III. With two maxima..... 4 cases.

One or more typical illustrations will be given for each group, and the character of the curves noted. In each case the poison used is indicated in parenthesis following the number of the curve, the first number expressing the number of grams of phenol and the second the number of grams of alcohol per 100 cc. of solution.

Fig. 1, *a* (0.650–0) illustrates an instance in which the distribution of resistance is quite uniform, that is, nearly the same numbers of organisms die in each equal interval of time. The survivor's curve in this case approximates a straight line, and the plot of the logarithms of the survivors is curved throughout its length. Apparently this is not the most common of distribution of resistances. It is evident that a distribution of this type is entirely out of harmony with the monomolecular concept. Fig. 1, *b* (0.580–4.75) illustrates a case in which the survivor plot is somewhat curved, but is far from logarithmic. Fig. 1, *c* (0.65–0) is interesting in that during the first two periods of time comparatively small numbers of cells stained, in later periods a larger and quite uniform number stained. The survivor's curve is therefore practically two straight lines. It is evident that in certain cases (4 among 27 studied) the yeast cells acquire the ability to stain in such a fashion that the survivor's curve approximates a straight line.

In Fig. 2 is given a series of three curves in which there is a distinct maximum during the first time period. Nine of the total of twenty-seven distributions are of this general type. Fig. 2, *a* (0.650–0) illustrates a survivor's curve which is an excellent approach to the logarithmic type; *i.e.*, the logarithms of the numbers (except at the end) plot as a straight line. In some cases, as shown in Fig. 2, *b*

(0.570–4.75) the survivor's curve is logarithmic for more than half its length, and practically linear for the remainder. Fig. 2, *c* (0.500–0) shows a survivor's curve with a double inflection; in this case there is no resemblance to a logarithmic curve. It is evident even in cases in which the maximum number die in the first time period the survivor's curve plotted may or may not be logarithmic.

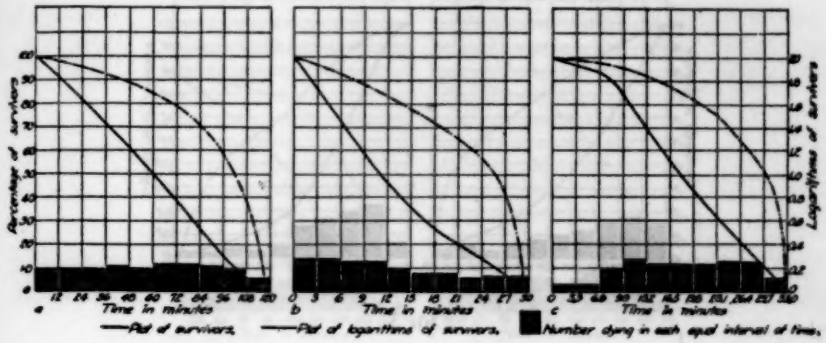

FIG. 1.

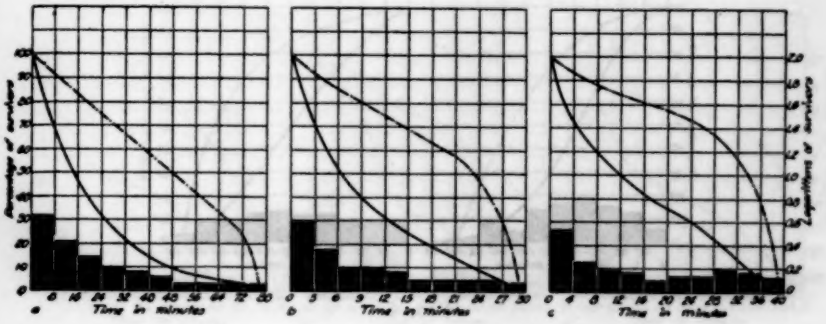

FIG. 2.

The two curves in Fig. 3 show distributions in which the maxima are reached in the second time period. It will be noted that the survivor's curves are sigmoid in shape and are not strictly logarithmic. In Fig. 3, *a* (0.650–0) a portion of the curve approximates a logarithmic curve. In Fig. 3, *b* (0.620–4.75) however, the approximation is even less evident.

Fig. 4 embodies results of the type secured when the frequency curve approaches a normal distribution curve. The best approximation is in Fig. 4, *a* (0.570–4.75). In this as in Fig. 4, *b* (0.650–0) the sigmoid character of the survivor's curve and lack of logarithmic character throughout its length are evident.

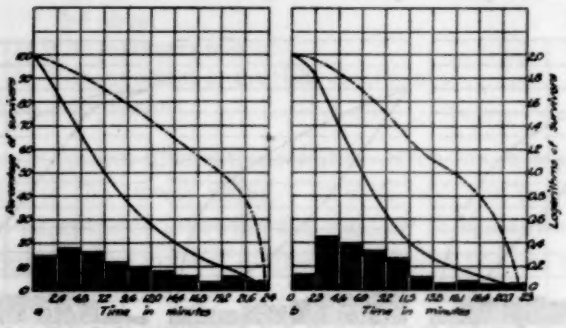

FIG. 3.

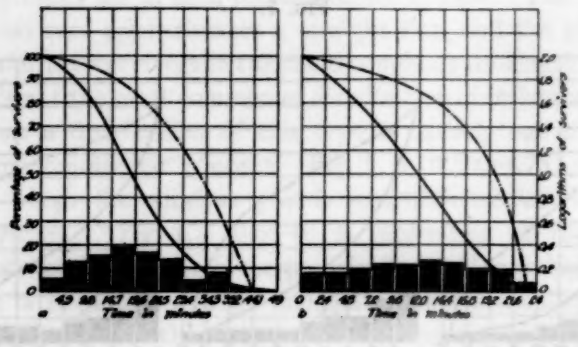

FIG. 4.

In three instances the single maximum was found to be well to the right of the middle of the rate curve. In Fig. 5 *a* (0.650–0) the part of the survivor's curve to the right of the maximum is approximately logarithmic, but the sigmoid character of the curve as a whole is evident in this as well as in Fig. 5, *b* (0.710–4.75).

In four instances two distinct maxima were to be noted; *i.e.*, the frequency curve was bimodal. Fig. 6, *a* (0.650–0) illustrates a case

in which the maxima were found in the first and seventh time periods. The survivor's curve here has three points of inflection. Fig. 6, *b* (0.650–0) shows a bizarre arrangement, the survivor's curve showing four points of inflection. Fig. 6, *c* (0.465–4.75) is interesting in that the maxima occur in the second and last periods and the survivor's shows two inflections.

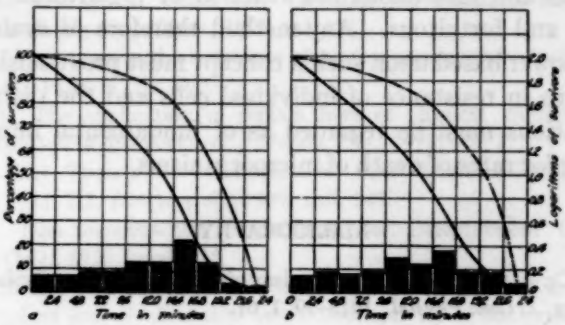

FIG. 5.

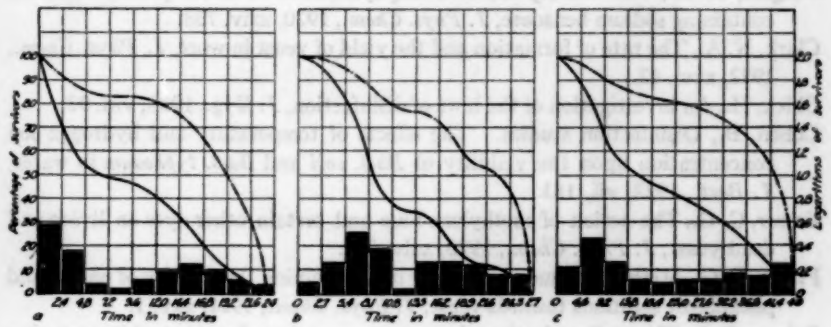

FIG. 6.

There is no obvious relationship between the type of distribution curve and the kind or concentration of poison used and the age of the culture. The concentration of yeast cells was the same in each case; *i.e.*, about 0.10 gm. of yeast per 100 cc.

SUMMARY AND CONCLUSIONS.

With yeast subjected to the action of phenol or of phenol and alcohol the curves of survivors as measured by resistance to staining with methylene blue when plotted against time do not in general prove to be logarithmic.

It is believed that such resemblances as have been found between such curves and monomolecular reaction or logarithmic curves are superficial and fortuitous. Any method therefore of evaluating disinfecting power based upon such a concept must prove misleading.

Variations in resistance of individual cells and the distribution of such variations must be regarded as of fundamental importance in accounting for rates of death of microorganisms.

BIBLIOGRAPHY.

Brooks, S. C., A theory of the mechanism of disinfection hemolysis and similar processes, *J. Gen. Physiol.*, 1918–19, 1, 61.

Brooks, S. C., Conductivity as a measure of vitality and death, *J. Gen. Physiol.*, 1922–23, v, 365.

Burgess, K. E., The toxicity towards staphylococcus of dilute phenol solutions containing sodium benzoate, *J. Phys. Chem.*, 1920, xxiv, 738.

Clark, N. A., The rate of formation and the yield of yeast in wort, *J. Phys. Chem.*, 1922, xxvi, 42.

Chick, H., An investigation of the laws of disinfection, *J. Hyg.*, 1908, viii, 92.

Cohen, B., Disinfection studies. The effects of temperature and hydrogen ion concentration upon the viability of *Bact. coli* and *Bact. typhosum* in water, *J. Bact.*, 1922, vii, 183.

Fraser, C. G., The action of methylene-blue and certain other dyes on living and dead yeast, *J. Phys. Chem.*, 1920, xxiv, 741.

Fraser, C. G., Methylene-blue as indicator in determining the toxicity of phenol and phenol-salt solutions towards yeast, *J. Phys. Chem.*, 1921, xxv, 1.

Fulmer, E. I., The acclimatization of yeast to ammonium fluoride and its reversion in wort, *J. Phys. Chem.*, 1921, xxv, 455.

Fulmer, E. I., The effect of the alcohol on the toxicity of phenol towards yeast, *J. Phys. Chem.*, 1921, xxv, 10.

Ikeda, K., Allgemeine Beziehungen zwischen Concentration und Giftwirkung der Quecksilberchloridlösungen, *Z. Hyg. u. Infectionskrankh.*, 1897, xxv, 95.

Lee, R. E., and Gilbert, C. A., Application of the mass law to the process of disinfection being a contribution to the "mechanistic theory" as opposed to the "vitalistic theory," *J. Phys. Chem.*, 1918, xxii, 348.

Loeb, J., and Northrop, J. H., On the influence of food and temperature upon the duration of life, *J. Biol. Chem.*, 1917, xxxii, 103.

Madsen, T., and Nyman, M., Zur Theorie der Desinfektion. I, *Z. Hyg. u. Infectionskrankh.*, 1907, lvii, 388.

Miller, W. L., Toxicity and chemical potential, *J. Phys. Chem.*, 1920, xxiv, 562.

Osterhout, W. J. V., Injury, recovery, and death, in relation to conductivity and permeability, Monographs on Experimental Biology, Philadelphia and London, 1922.

Paul, T., and Krönig, B., Ueber das Verhalten der Bakterien zu chemischen reagentien, *Z. physikal. Chem.*, 1896, xxi, 414.

Peters, R. A., Variations in the resistance of protozoon organisms to toxic agents, *J. Physiol.*, 1920–21, liv, 260.

Phelps, E. B., The application of certain laws of physical chemistry in the standardization of disinfectants, *J. Infect. Dis.*, 1911, viii, 27.

Reichel, H., Zur Theorie der Desinfektion. Die Desinfektionswirkung des Phenols. I, II, III, *Biochem. Z.*, 1909, xxii, 149.

Reichenbach, Die theoretischen Grundlagen der Desinfektion, *Centr. Bakt., Ite Abt., Orig.*, 1922, lxxxix, 15.

Shearer, C., Studies on the action of electrolytes on bacteria. Part I. The action of monovalent and divalent salts on the conductivity of bacterial emulsions, *J. Hyg.*, 1919–20, xviii, 337.

Smith, J. H., The killing of *Botrytis* spores by phenol, *Ann. Appl. Biol.*, 1921, viii, 27.

MEMBRANE POTENTIALS AND COLLOIDAL BEHAVIOR.

By A. V. HILL.

(From the Physiological Laboratory, University of Manchester, Manchester, England.)

(Received for publication, June 14, 1923.)

An answer has been given by Hitchcock[1] to a recent criticism by myself[2] of an argument vigorously exploited by Loeb in favor of his theory of colloidal behavior. Hitchcock suggests that this criticism rests upon a misunderstanding, but he admits the validity of my main contentions, and I am happy to note that Loeb has taken heed of them in two recent papers. I have no quarrel with many of the arguments put forward by Loeb, and in the first paragraph of my criticism I paid a tribute to the stimulating and convincing character of his studies. I have no alternative explanation to offer of the facts, though I recognise that alternative explanations of some of them are possible. What I attacked was the continual reiteration of an argument which is incorrect; *viz.*, that "the quantitative agreement between the observed P.D. and the P.D. calculated on the basis of the Donnan theory leaves little doubt that the observed P.D." (and therewith a variety of other colloidal phenomena) "is exclusively determined by the Donnan equilibrium." There may be perfectly good proofs that the Donnan equilibrium is the basis of the whole fabric of colloidal behavior, but this is not one of them. The agreement observed proves nothing but the second law of thermodynamics and is a necessary consequence of any theory which does not offend that law. Naturally, therefore, it can be deduced from the Donnan theory. Surely it is possible and expedient to criticise an argument which is false, without necessarily disagreeing with the theory which it was intended to support and being required to produce an alternative one. If it be not, then indeed my criticism was based on a misunderstanding.

[1] Hitchcock, D. I., *J. Gen. Physiol.*, 1922–23, v, 661.

[2] Hill, A. V., *Proc. Roy. Soc. London, Series A*, 1923, cii, 705.

MEMBRANE POTENTIALS AND COLLOIDAL BEHAVIOR.

Reply to the Note by Professor A. V. Hill.

By DAVID I. HITCHCOCK.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, June 25, 1923.)

There is no real point of conflict between our views and those expressed by Professor Hill. The mere agreement of the figures for P.D., considered abstractly and apart from experimental conditions, is not a proof of the existence of a Donnan equilibrium. For example, in an experiment in which the protein were omitted altogether there would also be agreement of the P.D. values, for each would be zero; but no one would think of calling this agreement in itself a proof of the existence of a Donnan equilibrium.

The statement to which Hill has objected was not intended to be taken out of its context and used alone as a complete argument. The proof of the agreement of the P.D. values was a necessary step in testing the theory. It shows, as Hill has admitted, that the systems studied were in equilibrium. The fact that they could be in equilibrium, and still have different hydrogen or chloride ion concentrations on the opposite sides of the membrane, was by no means obvious, and required explanation. Donnan's theory furnished this explanation; and it has been pointed out that the experiments are quantitatively in accord with other deductions from the theory.

Since Donnan's theory does explain the facts, and no other theory has been proposed which can explain them in the same quantitative manner, it seems necessary to retain Loeb's original conclusion that his data have proved the applicability of Donnan's theory to these experiments with proteins.

THE COMBINATION OF DEAMINIZED GELATIN WITH HYDROCHLORIC ACID.

By DAVID I. HITCHCOCK.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, May 28, 1923.)

I.

INTRODUCTION.

When a protein such as gelatin is treated with nitrous acid there is an evolution of nitrogen, presumably from the reaction of the nitrous acid with NH_2 groups in the protein. This reaction is the basis of the well known Van Slyke[1] method for the quantitative determination of free amino groups. The resulting deaminized proteins, in which each NH_2 group may be assumed to have been replaced by OH, were studied by Skraup,[2] who investigated in some detail the products of their hydrolysis. Deaminized gelatin was prepared according to Skraup's method by Blasel and Matula,[3] who showed by hydrogen electrode measurements that the deaminized protein was still capable of combining with hydrochloric acid.

The present work was undertaken to compare the maximal combining capacity for hydrochloric acid of gelatin with that of deaminized gelatin. If the combination of these proteins with hydrochloric acid is true chemical combination, and if the process of deaminization consists simply in replacing NH_2 groups by OH, then it would be expected that the difference in their combining capacities should be chemically equivalent to the NH_2 groups removed. That this expectation has been confirmed is shown by the following experimental results.

[1] Van Slyke, D. D., *J. Biol. Chem.*, 1911, ix, 185; 1912, xii, 275.

[2] Skraup, Z. H., *Monatsh. Chem.*, 1906, xxvii, 653; 1907, xxviii, 447.

[3] Blasel, L., and Matula, J., *Biochem. Z.*, 1913–14, lviii, 417.

II.

The Deaminizing Reaction.

It was found by Van Slyke[1] that the deaminizing reaction took place rapidly and quantitatively if the amino substance was treated with a great excess of a solution containing equivalent amounts of sodium nitrite and acetic acid. The preparation of Skraup was obtained under similar conditions, but after allowing the mixture to stand over night he heated the acid solution for 2 hours on a water bath and then precipitated the deaminized gelatin repeatedly by saturation with ammonium sulfate.

In order to determine whether this treatment caused any further reaction than that occurring in the Van Slyke apparatus, measurements were made of the amino nitrogen in gelatin, by Van Slyke's[1] method, and of the total nitrogen in gelatin and in deaminized gelatin, by the Kjeldahl method.[4] Three lots of deaminized gelatin were used. No. 1 was prepared from Cooper's commercial gelatin according to Skraup, except that it was precipitated only once by ammonium sulfate, while No. 2 was part of the same lot which was not heated on the water bath and not precipitated, but was dialyzed under pressure in collodion cylinders after standing over night in the nitrous acid solution. The dialysis was started in running tap water; after about 4 days the contents of the bags were brought to pH 3.6 with hydrochloric acid, and then dialyzed for a week against repeated changes of hydrochloric acid of pH 3.6; finally the bags were kept for 3 days in repeated changes of distilled water. This reduced the specific conductivity of the resulting 3 per cent solutions to less than 10^{-4} reciprocal ohms. The concentrations of these solutions were determined by drying 25 cc. samples to constant weight at 110°C., and 25 cc. samples were likewise used for the Kjeldahl determinations. The gelatin analyzed was a solution of isoelectric gelatin which had been prepared from Cooper's gelatin as described by Loeb.[5] Preparation 3 of deaminized gelatin was made by dialyzing the residues

[4] Sherman, H. C., Methods of organic analysis. New York, 2nd edition, 1912, 291.

[5] Loeb, J., Proteins and the theory of colloidal behavior, New York and London, 1922, 35.

from the Van Slyke determinations, for which the initial material was isoelectric gelatin.

By the Van Slyke method the following figures were found for the percentage of amino nitrogen, using samples containing from 0.8 to 1.2 gm. of dry gelatin: 0.525, 0.505, 0.582, 0.662, 0.540, 0.514; average, 0.555 per cent. This agrees with the results of Van Slyke and Birchard,[6] who found 3.12 per cent of the total nitrogen in gelatin to be amino nitrogen; taking the total nitrogen as 17.96 per cent (see below), their figure for amino nitrogen becomes 0.560 per cent of the gelatin. Similar figures were obtained by Northrop,[7] who found the normality of 1 per cent gelatin to be 0.0036 or 0.0038 with respect to NH_2 groups; accordingly his value for the percentage of amino nitrogen in dry gelatin is $10 \times 0.0037 \times 14.01 = 0.519$ per cent.

By the Kjeldahl method the following figures for total nitrogen were obtained.

Gelatin	18.02	17.94	17.97	17.89	average,	17.96	per cent.
Deaminized gelatin No. 1	16.98	16.94	16.99		"	16.97	" "
" " " 2	17.46	17.35	17.41	17.37	"	17.40	" "

It is evident that the difference between the figure for gelatin and that for deaminized gelatin No. 2 is almost exactly equal to the percentage of amino nitrogen removed in the Van Slyke analysis. In the case of deaminized gelatin No. 1, which was prepared according to Skraup, with heating, the loss of nitrogen is considerably greater, indicating that a more extensive reaction has taken place than the simple removal of free amino groups. This was corroborated by the fact that in the dialysis of No. 1 much more material was lost by diffusion through the collodion than was the case with No. 2.

These figures may be summarized as follows:

1 gm. gelatin	= 0.00555	gm. or	0.000396	equivalents	amino N.
1 " "	= 0.1796	" "	0.01282	"	total "
1 " deaminized gelatin No. 2	= 0.1740	" "	0.01242	"	" "
Loss in deamination	= 0.0056	" "	0.00040	"	N.

Equal weights of gelatin and deaminized gelatin are taken as chemically equivalent, because the formula weight of the group OH, 17, is so close

[6] Van Slyke, D. D., and Birchard, F. J., *J. Biol. Chem.*, 1913–14, xvi, 539.

[7] Northrop, J. H., *J. Gen. Physiol.*, 1920–21, iii, 715.

to that of the NH_2 group, 16, and because the groups affected are such a small part of the weight of the protein. These results indicate that if the preparation of deaminized gelatin is carried out without heating, the reaction is simply a replacement of NH_2 by OH, as in the case of ordinary aliphatic amines.

This simple conception of the reaction occurring in the preparation of deaminized gelatin was confirmed by analyzing the product by the Van Slyke method; the percentage of amino nitrogen found was 0.025 for Preparation 1 and 0.029 for Preparation 2. It is believed that these figures should both be zero, since the volumes of nitrogen obtained, after correction for the blank from the reagents, were only 0.16 and 0.15 cc. The absence of amino nitrogen in several other preparations of deaminized gelatin, prepared both with and without heating, was further confirmed by the formol titration method of Sörensen.[8] In no case was there any difference between the titrations with and without formaldehyde, if proper correction was made for the acidity of the formaldehyde solution itself. It may be stated that this is quite at variance with the results reported by Herzig and Lieb,[9] who found over twice as much amino nitrogen in gelatin as any of the authors quoted above, and found similar high figures for amino nitrogen in deaminized gelatin, even after it had been twice deaminized.

III.

Combination of Deaminized Gelatin with HCl.

In order to calculate the combining capacity of deaminized gelatin for acid, it was necessary to know the isoelectric point of the material. In the work of Blasel and Matula[3] this was not considered, and their curve therefore does not represent the true amounts of combined acid.

The isoelectric point of a protein has been shown by Loeb[10] to coincide with a minimum of osmotic pressure. Accordingly measurements were made of the osmotic pressure at 24°C. and of the pH of the

[8] Sörensen, S. P. L., *Compt. rend. trav. Lab. Carlsberg*, 1907, vii, 1; *Biochem. Z.*, 1908, vii, 64.

[9] Herzig, J., and Lieb, H., *Z. physiol. Chem.*, 1921, cxvii, 1.

[10] Loeb, J., Proteins and the theory of colloidal behavior, New York and London, 1922, 37.

protein solutions at equilibrium, in the manner described by Loeb. The results obtained with 1 per cent solutions of Preparation 1, and other preparations which had been heated even more than 2 hours on

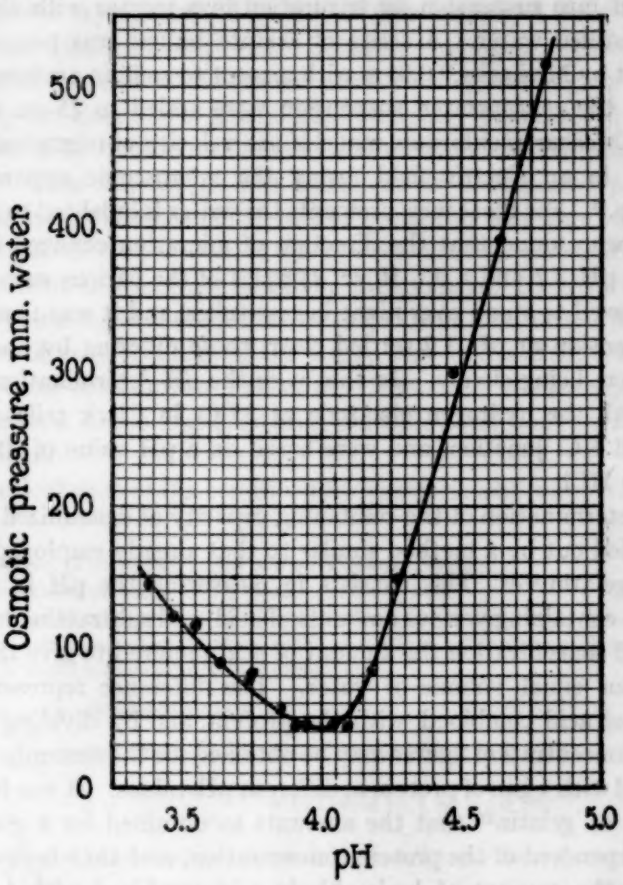

FIG. 1. Effect of pH on osmotic pressure of 1 per cent deaminized gelatin.

the water bath, showed a minimum of osmotic pressure at pH 3.5 to 3.8. Preparation 2, however, exhibited a minimum of osmotic pressure at pH 4.0, as is shown by Fig. 1.

This location of the isoelectric point was confirmed in another way in the case of Preparation 3, which was also prepared without heating. The protein was coated on particles of collodion by mixing 50 cc. of a

0.1 per cent solution with 50 cc. of a suspension of collodion particles, prepared as described by Loeb,[11] and allowing the mixture to stand over night. The mixture was then centrifuged, and the sediment was again put into suspension by trituration in a mortar with about 15 cc. of distilled water. A series of acetate buffers was prepared as described by Michaelis,[12] 0.01 M with respect to sodium acetate, and 5 drops of the concentrated suspension were added to 25 cc. of each buffer. Measurements were made of the velocity of migration of the particles in an electric field, using the microscopic apparatus of Northrop.[13] For these measurements the writer is indebted to Mr. M. Kunitz, who found that the direction of migration changed its sign between pH 3.7 and 4.0. 10 cc. samples of the various suspensions were allowed to stand over night in test-tubes, and it was found that the suspension at pH 3.9 settled clear, those differing by about pH 0.1 or more being cloudy. In this work the pH determinations were made with the hydrogen electrode at 33°C. in Clark cells with a saturated KCl junction, and were based on a pH value of 1.037 for 0.1000 M HCl.

The determination of the combining capacity of deaminized gelatin was carried out by a method similar to that already employed in the case of gelatin.[14,15] This consists in measuring the pH in protein solutions containing various amounts of acid, and subtracting from the total acid concentration the amount of acid required to give the same pH to an equal volume of water. The difference represents the amount of acid combined with the protein, and by dividing by the protein concentration figures may be obtained for the amounts of acid combined with 1 gm. of protein at different pH values. It was found in the case of gelatin[15] that the amounts so obtained for a given pH were independent of the protein concentration, and that between pH 1 and 2 the amount of hydrochloric acid combined with 1 gm. of gelatin was constant.

[11] Loeb, J., *J. Gen. Physiol.*, 1922–23, v, 109.

[12] Michaelis, L., Die Wasserstoffionenkonzentration, Berlin, 1914, 184.

[13] Northrop, J. H., *J. Gen. Physiol.*, 1921–22, iv, 629.

[14] Loeb, J., Proteins and the theory of colloidal behavior, New York and London, 1922, 51.

[15] Hitchcock, D. I., *J. Gen. Physiol.*, 1921–22, iv, 733.

In the case of deaminized gelatin the method was modified by performing the titration with a single sample of protein, instead of making up a fresh sample to the same volume for each pH determination. A vessel of the type described by Bovie[16] was used, with an electrode of platinized platinum wire and bubbling hydrogen. Contact was made with a saturated potassium chloride bridge through a tube not quite tightly closed by a ground glass stopper. Junctions of this type were tested by Lamb and Larson[17] and recommended by LaMer and Baker.[18] The salt bridge was connected to a saturated potassium chloride calomel cell, and the whole apparatus was kept in a water bath at 33° ± 0.1. 50 cc. of the protein solution were placed in the cell, and after the electrode had come to equilibrium the titrations were made by running in standard HCl solution from a burette. After each addition of acid, readings of the E.M.F. were taken at intervals until they became constant for 2 minutes. The E.M.F. measurements were made with a Leeds and Northrup portable potentiometer reading to 0.5 millivolt.

The calculation of the combined hydrochloric acid was performed as follows:

Let a = No. of cc. of HCl added to e cc. of protein solution.
b = concentration of HCl in burette, mols per liter.
c = initial concentration of protein solution, gm. per liter.
d = concentration of HCl having the same pH in water alone, mols per liter.
e = initial volume of protein solution, cc.

Then $a + e$ = volume of mixture, cc.

$\frac{ab}{a+e}$ = concentration of HCl in mixture, mols per liter.

$\frac{ab}{a+e} - d = \frac{ab - ad - ed}{a+e}$ = combined HCl, mols per liter.

$\frac{ec}{a+e}$ = concentration of protein in mixture, gm. per liter.

$\frac{ab - cd - ed}{ec}$ = mols of HCl combined with 1 gm. of protein.

The values of d were obtained from the curve constructed in connection with the previous work on gelatin;[15] this curve was checked by titrating 50 cc. of water with 0.201 M HCl in the new apparatus. In case the

[16] Bovie, W. T., *J. Am. Chem. Soc.*, 1922, xliv, 2892.
[17] Lamb, A. B., and Larson, A. T., *J. Am. Chem. Soc.*, 1920, xlii, 229.
[18] LaMer, V. K., and Baker, L. E., *J. Am. Chem. Soc.*, 1922, xliv, 1954.

original protein solution is not at the isoelectric point, the values for the combined acid must be corrected by adding or subtracting a constant amount to make the point of zero combination coincide with the isoelectric point. The only assumptions involved in the calculation are those used previously; namely, that the same concentration of uncombined HCl is required to give the same pH to equal volumes of water and of protein chloride solution, and that there is no acid combined with the protein at its isoelectric point.

TABLE I.

Titration of Deaminized Gelatin with Hydrochloric Acid.

A		B		C		D	
HCl	pH	HCl	pH	HCl	pH	HCl	pH
cc.		*cc.*		*cc.*		*cc.*	
0	3.88	0	4.02	0	4.02	1.20	3.61
0.50	3.18	0.94	3.66	1.29	3.53	1.90	3.35
0.99	2.78	2.81	2.94	2.94	2.88	2.80	2.99
1.99	2.29	4.58	2.32	4.92	2.21	4.40	2.45
3.17	2.03	6.58	2.00	5.93	2.02	7.10	1.98
4.51	1.85			6.93	1.89		
				8.43	1.75		

A. Preparation 3. Initial volume, e = 50 cc. Concentration of protein, c = 6.99 gm. per liter. Concentration of HCl, d = 0.201 M.

B and C. Preparation 2. Initial volume, e = 50 cc. Concentration of protein, c = 29.69 gm. per liter. Concentration of HCl, d = 0.201 M.

D. Preparation 2. In this case the solutions were made up to constant volume before measuring pH. The figures are cc. of 0.1003 M HCl contained in 35 cc. of solution containing also 0.743 gm. of deaminized gelatin.

The results of the titration experiments are given in Table I. From these results the amounts of hydrochloric acid combined with 1 gm. of protein were calculated in the way just described; the quantities so obtained are plotted in Fig. 2. The correction mentioned was necessary only in the case of Experiment A; in the other cases the solution happened to be at the isoelectric point when the dialysis was stopped. The curve indicates that deaminized gelatin has a maximum combining capacity of 0.00044 mols of HCl per gm. of protein.

IV.

CONCLUSIONS.

In a previous paper[15] it was stated that 1 gm. of gelatin could combine with 0.00092 mols of HCl. This figure should be corrected by subtracting the amount of acid necessary to shift the zero point from pH 4.78 to 4.70, the isoelectric point of gelatin. This correction makes the value for the maximum combined HCl 0.00089 mols per gm. of gelatin, and changes the combining weight of gelatin to about 1,120.

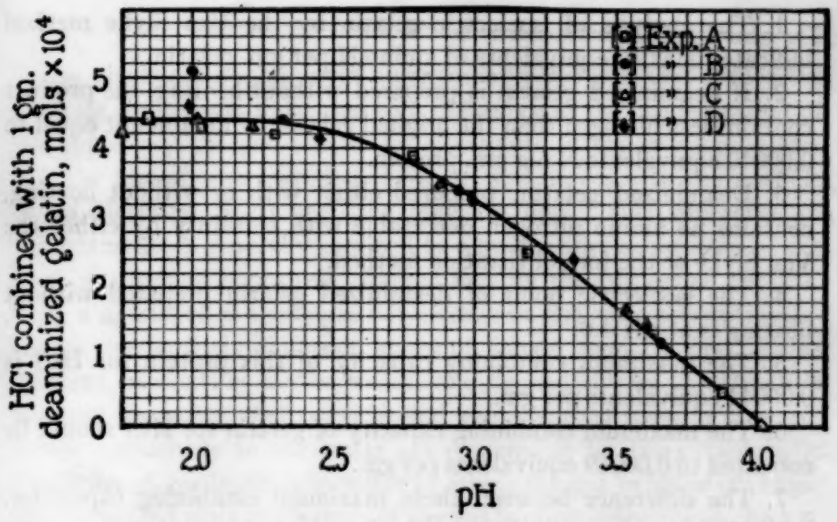

FIG. 2. Combination of deaminized gelatin with HCl.

The number of equivalents of amino nitrogen removed in deaminizing 1 gm. of gelatin was found to be 0.00040, and this was checked by the loss in total nitrogen. The difference between the maximum combining capacities for HCl of 1 gm. of gelatin and 1 gm. of deaminized gelatin is 0.00089 − 0.00044 = 0.00045. Considering the limitations of the methods which had to be employed to get these figures, the agreement is good.

This means, then, that the loss in combining capacity for HCl suffered by 1 gm. of gelatin in being deaminized is chemically equivalent to the number of amino groups removed. The combining

capacity for HCl still retained by deaminized gelatin is presumably to be ascribed to the NH groups which are not attacked by HNO_2. For each atom of nitrogen lost in the deaminizing reaction, the protein loses the capacity to combine with one hydrogen ion. Therefore the present work constitutes a new indication of the truly chemical nature of the combination between protein and acid.

V.

SUMMARY.

1. The analysis of isoelectric gelatin by the Van Slyke method indicates 0.00040 equivalents of amino N per gm. gelatin.

2. If deaminized gelatin is prepared without heating, the product contains less nitrogen than the original gelatin by an amount equal to 0.00040 equivalents N per gm. protein.

3. Deaminized gelatin, prepared either with or without heating, contains no amino nitrogen detectable with certainty by either the Van Slyke or the formol titration method.

4. The isoelectric point of deaminized gelatin prepared without heating is at pH 4.0.

5. The maximum combining capacity of this protein for HCl is 0.00044 equivalents per gm.

6. The maximum combining capacity of gelatin for HCl should be corrected to 0.00089 equivalents per gm.

7. The difference between these maximum combining capacities, 0.00045, is nearly equivalent to the loss in amino or total nitrogen occurring in the deaminizing reaction.

8. This equivalence constitutes a new indication that the combination of protein with acid is chemical combination.

The writer is indebted to Dr. Jacques Loeb for suggesting this work.

ON THE LOCATION OF THE FORCES WHICH DETERMINE THE ELECTRICAL DOUBLE LAYER BETWEEN COLLODION PARTICLES AND WATER.

By JACQUES LOEB.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, July 12, 1923.)

I.

The electrical double layer surrounding solid particles suspended in water is assumed to be due to a difference in the concentration of the oppositely charged ions of an electrolyte in the enveloping film (*i.e.* the film of water which adheres to and moves with the particle in an electrical field) and the opposite film. This difference in concentration is generally ascribed to a preferential adsorption of one kind of a pair of oppositely charged ions by the suspended particle. Such a definition is inadequate inasmuch as the term adsorption includes two entirely different kinds of forces; namely, first, forces inherent exclusively in the water, and second, forces of attraction between the suspended particle and the adsorbed ions. Forces inherent exclusively in the water are responsible for the cataphoretic migration of gas bubbles in water in which the gas bubbles are negatively charged since the nature of the gas is of no influence on the P.D. McTaggart[1] suggested that in this case there exists an excess of OH ions in the film of water which envelops the gas bubble (adhering to and moving with it); while the opposite film has an equal excess of H ions. The writer has recently suggested that this electrical double layer might be due to an orientation of the molecules of water at the surface of the water whereby the oxygen atoms of the water molecules are turned outwards and the two hydrogen atoms or ions are turned inside into the water.[2] Such an orientation of the molecules of water at the free surface might also explain the well known fact that the

[1] McTaggart, H. A., *Phil. Mag.*, 1914, xxvii, 297; xxviii, 367; 1922, xliv, 386.

[2] Loeb, J., *J. Gen. Physiol.*, 1922–23, v, 513.

smallest particles torn off mechanically from the surface of water are negatively charged, since it would be likely that if fine particles are torn off from the surface they would carry an excess of OH ions with them, leaving behind an excess of H ions. It is, however, possible that the anion responsible for the negative charge in these cases might not have been the OH ion but the anion of an electrolyte present in small traces. Our recent experiments have led us to suspect that such impurities play a greater rôle in the cataphoretic P.D. of solid particles in "pure water" than has been realized. As has been pointed out by Lenard[3] and McTaggart, there can be no doubt that the electrical double layer is in these cases determined entirely by forces inherent in the water itself. There is no reason why these forces should not also influence the cataphoretic P.D. at the boundary of solid particles and water.

The second source of an unequal distribution of a pair of oppositely charged ions at the boundary of suspended particles and water are forces inherent in the suspended particles whereby ions of a definite sign, either positive or negative, are attracted by the particles. We have a clear case of the action of such forces when a substance dissolved in the water, *e.g.* a protein, forms a durable irreversible film on the surface of the particle.

II.

The influence of ordinary electrolytes, such as HCl, H_2SO_4, NaOH, NaCl, $CaCl_2$, $LaCl_3$, Na_2SO_4, and $Na_4Fe(CN)_6$, on the cataphoretic P.D. of collodion particles in water has already been described,[4] but for the convenience of the reader Figs. 1 and 2 are reproduced from a preceding paper, illustrating the effect of the electrolytes mentioned on the cataphoretic P.D. of collodion particles.

Fig. 1 shows the effect of different concentrations of HCl, H_2SO_4, and NaOH on the cataphoretic P.D. of collodion particles measured by microscopic observation of the rate of migration of the particles as described in a previous paper,[4] and calculated from these measurements with the Helmholtz-Perrin formula (multiplying by 14 the

[3] Lenard, P., *Ann. Physik*, 1915, xlvii, 463.
[4] Loeb, J., *J. Gen. Physiol.*, 1922–23, v, 109.

velocity of migration in cm.$^{-4}$ for a gradient of 1 volt per cm. at 20°C. The distilled water, before acid or alkali was added, had a pH of about 5.8 and the cataphoretic P.D. of the particles was about 30 millivolts,

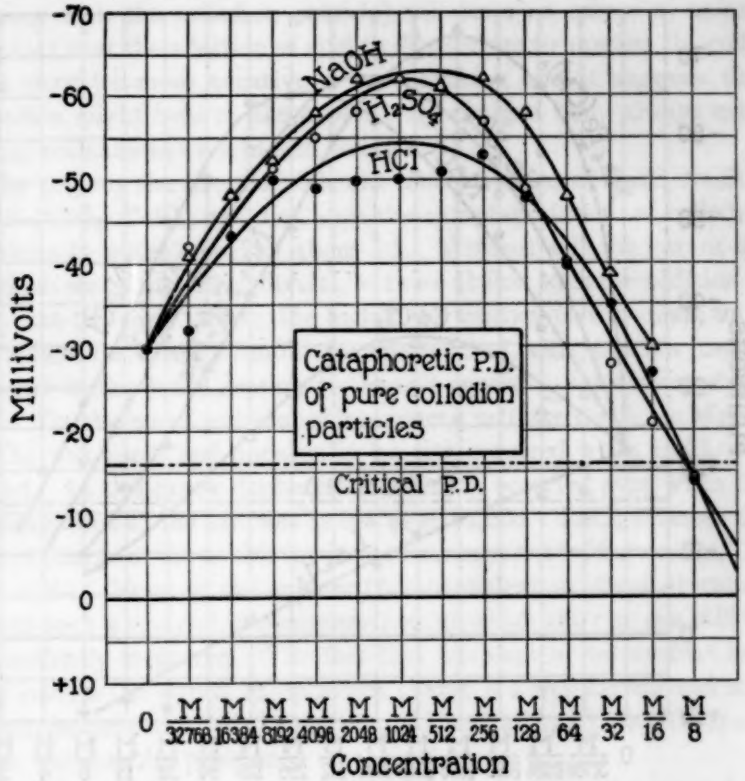

FIG. 1. Influence of acid and alkali on the cataphoretic P.D. between collodion particles and water. The original pH of water was about 5.0. The abscissæ are the concentrations of acids or alkali, the ordinates are the P.D. in millivolts. The collodion particles were negatively charged. The line marked "Critical P.D." (at 16 millivolts) is the P.D. below which the collodion suspension is no longer stable.

the particles being negatively charged. The addition of either acid or alkali increased this negative charge until a maximum was reached which was slightly higher for NaOH than for HCl, being about 65

millivolts in the case of NaOH and about 55 millivolts in the case of HCl. This maximum was reached when the concentration of acid or alkali was between M/1,000 and M/500. A further increase in concentration of the acid or alkali depressed the P.D. again.

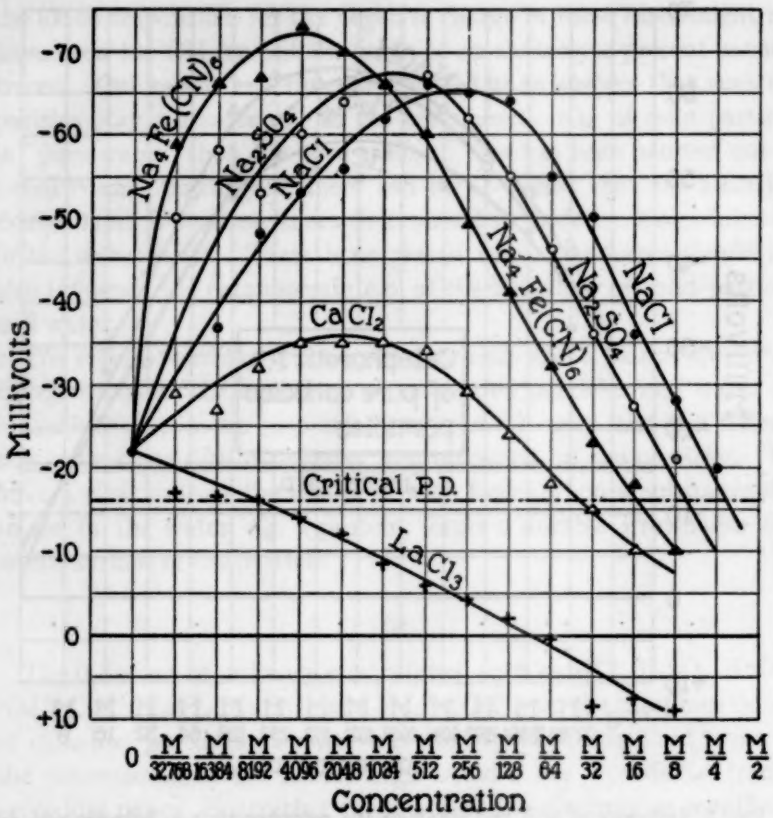

FIG. 2. Influence of $Na_4Fe(CN)_6$, Na_2SO_4, NaCl, $CaCl_2$, and $LaCl_3$ on the P.D. at pH 5.8. Addition of little salt with monovalent cation raises the P.D. to about 70 millivolts and the more rapidly the higher the valency of the anion. With $CaCl_2$ only a slight rise and with $LaCl_3$ no rise occurs in the concentrations used. In concentrations above M/64 $LaCl_3$ causes a reversal of the sign of charge of the particles.

What interests us here are the following two facts: that the collodion particles are always negatively charged and that the addition of acid or alkali at first increases the P.D. and the more so the higher the con-

centration of the acid or alkali added, as long as the concentration remains below a concentration somewhere between M/1,000 and M/500. This means that no matter whether we add acid or alkali to water of pH 5.8, the enveloping film of water (adhering to and moving with the collodion particle) will have an excess of anions. The fact that the addition of acid to distilled water renders the collodion particles more negative is very striking and it suggests that collodion might behave like a positively charged body always combining with anions even in acid solution.

Fig. 2 gives the effect of different concentrations of NaCl, Na_2SO_4, $Na_4Fe(CN)_6$, $CaCl_2$, and $LaCl_3$ on the cataphoretic P.D. of collodion particles in water at pH of about 5.8. Without salt the P.D. of the particles was about 22 millivolts, but rose at first with the addition of salt, and the more rapidly the higher the valency of the anion, until a maximum of about 70 millivolts was reached, after which a further increase in the concentration of the salt caused a diminution of the P.D. The rise was considerably less when a salt like $CaCl_2$ (or $MgCl_2$, $BaCl_2$) was used, and apparently no rise occurred when $LaCl_3$ was added. In this case a depression of the P.D. occurred even when the concentration of the salt was as low as M/32,000. $LaCl_3$ reverses the sign of charge of the double layer at a concentration of between M/128 and M/64. None of the salts with monovalent or divalent cation causes such a reversal in concentrations at which the P.D. can still be conveniently measured. The fact that trivalent or tetravalent ions may reverse the sign of cataphoretic charge is a well known and universal phenomenon. McTaggart found that $LaCl_3$ and $Th(NO_3)_4$ even make gas bubbles positive.[1]

The fact which interests us in Fig. 2 is that salts with monovalent and divalent cations at first raise the negative charge of the particles in cataphoresis (that is, as long as the concentration of the salts does not exceed a certain value). It may be mentioned incidentally that LiCl and KCl act on the cataphoretic P.D. almost quantitatively like NaCl. Since in all these cases the enveloping film has an excess of anions which at first increases with the concentration of the electrolytes ($LaCl_3$ or $ThCl_4$ excepted), it again looks as if the excess of anions in the film might be due to a preferential attraction of anions by the collodion particle. With the aid of dyes and proteins it can

be shown, however, that cations of these latter substances can form durable films on collodion, but that the anions cannot do so. When collodion particles are put into a solution of an acid or basic dye, it is found that at pH 5.8 they remove the dye from the solution and are themselves stained correspondingly when the dye is basic but less or not at all when it is acid. They thus "adsorb" dye cations, *e.g.*, basic fuchsin, neutral red, malachite green, but not dye anions, *e.g.* acid fuchsin, methyl orange, or congo red. When solutions of gelatin or albumin are used, the collodion particles are covered with a film of gelatin or of crystalline egg albumin when the protein is a cation (*e.g.* in the case of albumin chloride), but not when the protein is an anion (*e.g.* in the case of Na albuminate). This suggests that the increase in the negative charge of the collodion particles caused by the addition of ordinary crystalloidal electrolytes, as expressed in Figs. 1 and 2, cannot with any degree of certainty be attributed to the chemical forces inherent in the collodion, but must be attributed to forces inherent in the water, unless further evidence to the contrary is furnished.

III.

Solutions of six dyes were prepared in water of pH 5.8 in concentrations varying from M/2,000,000 to about M/256, *i.e.* those concentrations in which it was possible to measure the rate of motion of the particles in the solution of the dye with the aid of dark field illumination.

Fig. 3 gives the results. A comparison of Figs. 2 and 3 suggests that the salts of the three acid dyes, congo red, methyl orange, and acid fuchsin, act like NaCl or $Na_4Fe(CN)_6$; namely, the addition of the dye raises at first the cataphoretic P.D. until a maximum is reached which lies at a molecular concentration of the dye between M/4,096 and M/1,000; after which a further increase in the concentration of the dye causes a diminution of the P.D. The ions of the dye are in this case anions which cannot form a film on the surface of the collodion particles.

The influence of the three basic dyes on the cataphoretic P.D. is entirely different. These dyes do not raise the cataphoretic P.D. of the collodion particles but depress it so powerfully that in concentra-

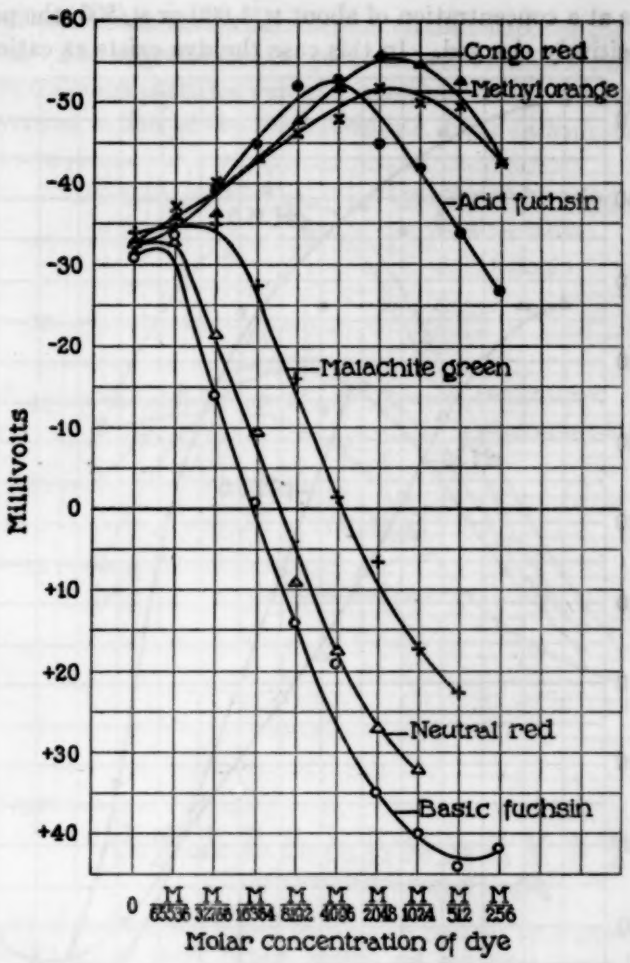

FIG. 3. Influence of dye salts on the cataphoretic P.D. of collodion particles originally of pH 5.8. Basic dyes (basic fuchsin, neutral red, and malachite green) depress the P.D. and reverse its sign at low concentrations; acid dyes (acid fuchsin, methyl orange, and congo red) act in the way of ordinary salts like NaCl (see Fig. 2).

tions between M/16,000 and M/4,000 the sign of the charge of the particles is already reversed, the enveloping film of water assuming a positive charge. In the case of basic fuchsin the P.D. becomes 40

millivolts at a concentration of about M/1,000 or M/500, the particles being positively charged. In this case the dye exists as cations and

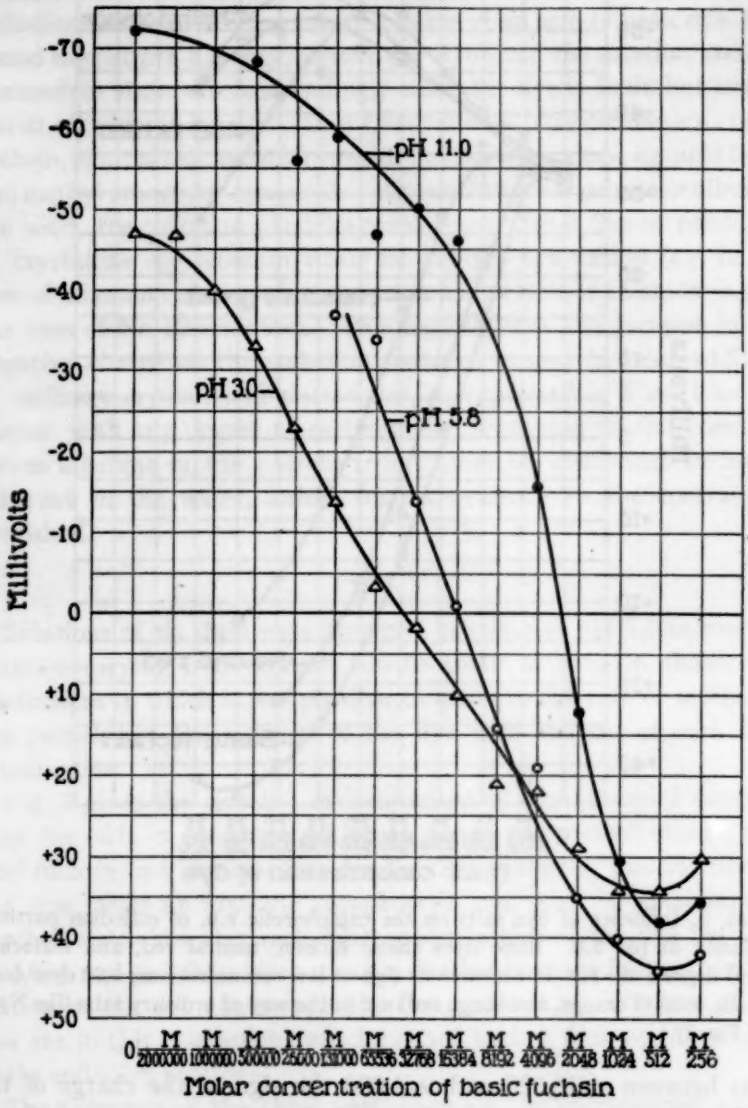

FIG. 4. A basic dye (basic fuchsin) depresses the P.D. of collodion particles and reverses its sign not only at pH 5.8 but also at 3.0 and 11.0.

these cations form a visible durable film on the surface of the collodion particles. The migration of the particles was measured immediately after the collodion particles were put into these dyes. The depressing and reversing action of the basic dyes, *e.g.* basic fuchsin, occurs not

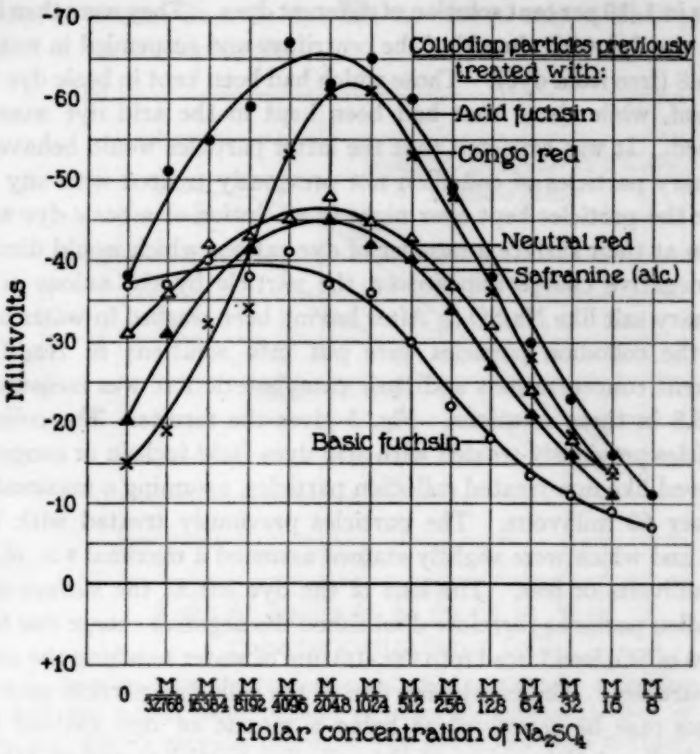

FIG. 5. Proof that the film formed on collodion particles by a previous treatment with dye cations (basic fuchsin, neutral red, safranine) lowers the effect of Na_2SO_4 on the P.D., while the effect of this salt on the P.D. of collodion particles previously treated with dye anions (acid fuchsin, congo red) is the same as upon non-treated particles. In acid dyes no film of dye is formed on the particles.

only when the basic dye is dissolved in ordinary distilled water at pH 5.8 but also when it is dissolved in N/1,000 HCl or N/1,000 NaOH (*e.g.* at pH about 3.0 and 11.0) as shown in Fig. 4. The concentration at which the dye reverses the sign of charge of the particles increases with the pH.

The basic dyes form a more or less durable film on the surface of the collodion particles and it seemed of interest to study to what extent such a film might alter the influence of ordinary salts on the cataphoretic P.D. of the particles. Collodion particles were kept for 24 hours in 1/10 per cent solution of different dyes. They were then freed from the dye with the aid of the centrifuge and suspended in water of pH 5.8 (free from dye). Those which had been kept in basic dye were stained, while those that had been kept in the acid dye were not stained. It was expected that the latter particles would behave like ordinary particles of collodion not previously treated with any dye; while the particles kept over night in a solution of a basic dye would retain at their surface a number of dye cations which would diminish the negative charge imposed on the particle by the anions of any ordinary salt like Na_2SO_4. After having been washed in water of pH 5.8, the collodion particles were put into solutions of Na_2SO_4 of different concentrations and their cataphoretic P.D. was measured at pH 5.8 in these solutions. Fig. 5 gives the results. The collodion particles previously treated with acid dyes (acid fuchsin or congo red) behaved like non-treated collodion particles, assuming a maximal P.D. of over 60 millivolts. The particles previously treated with basic dyes and which were slightly stained assumed a maximal P.D. of only 45 millivolts or less. The ions of the dye left at the surface of the collodion particles therefore diminished the negative charge due to the excess of SO_4 ions forced into the stratum of water touching the collodion particles. The enveloping film of the collodion particle may then in this case be conceived as being a mosaic of dye cations (held chemically at the surface of the collodion particles) and of SO_4 and possibly OH ions driven into the enveloping film by forces inherent in the water.

The experiment was repeated with $CaCl_2$ as the testing salt and the same difference was found. The P.D. of the collodion particles previously treated with basic fuchsin was depressed more by $CaCl_2$ than the P.D. of the particles previously treated with acid fuchsin (Fig. 6).

It is, however, worthy of notice that the enveloping film did not assume a positive charge at pH 5.8 when the collodion particles had previously been stained with a basic dye; and furthermore, it is worthy

of notice that in Fig. 5 the character of the curves expressing the influence of Na_2SO_4 on the cataphoretic P.D. is the same in the stained and the non-stained particles of collodion. The only difference is a slight diminution of the P.D. in the stained particles. One might be tempted to assume that the dye cations formed only a small part of the area of the enveloping film.

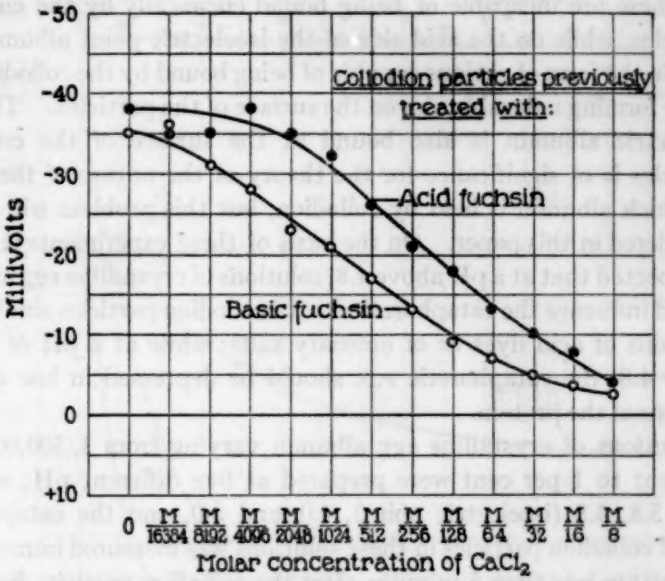

FIG. 6. Proof that the film formed on collodion particles by a previous treatment with dye cations (basic fuchsin) increases the depressing effect of $CaCl_2$ on the P.D., while a previous treatment with dye anions (acid fuchsin) has no such effect.

IV.

The influence of protein salts on the cataphoretic P.D. of collodion is of special interest. The isoelectric point of crystalline egg albumin is at pH 4.8, and it was shown in a preceding publication that a protein film is formed on the surface of collodion particles kept over night in a weak solution of isoelectric albumin.[5] These albumin-covered collodion particles were separated from the protein solution with the centrifuge and were then transferred into water of different pH to test the effect of electrolytes on their cataphoretic P.D. It was found

[5] Loeb, J., *J. Gen. Physiol.*, 1922–23, v, 479.

that at pH 4.8 or below, electrolytes influenced the particles as if they consisted of egg albumin, while for pH > 4.8, *i.e.* on the alkaline side of the isoelectric point of albumin, the particles behaved as if the albumin had been dissolved and as if only uncoated collodion particles were left. In the light of the experiments with dyes these results seem clear. At a pH above 4.8 the albumin forms only anions and these are incapable of being bound chemically by the collodion particles, while on the acid side of the isoelectric point albumin ions exist in the form of cations capable of being bound by the collodion and hence forming a durable film on the surface of the particles. That the isoelectric albumin is also bound at the surface of the collodion particles is of significance for the theory of the nature of the forces by which albumin is held by collodion, but this problem will not be considered in this paper. On the basis of these experiments it was to be expected that at a pH above 4.8, solutions of crystalline egg albumin should influence the cataphoretic P.D. of collodion particles similarly to solutions of acid dyes or of ordinary salts; while at a pH of 4.8, or below 4.8, the cataphoretic P.D. should be depressed in low concentrations of the protein.

Solutions of crystalline egg albumin varying from 1/500,000 of 1 per cent to 1 per cent were prepared at five different pH; namely, 11.0, 5.8, 4.8 (isoelectric point), 4.0, and 3.0, and the cataphoretic P.D. of collodion particles in these solutions was measured immediately (*i.e.* within less than 5 minutes after the collodion particles had been put into the solutions). Only the experiments with concentrations up to 1/16 per cent could be used without making a correction for the increase in the viscosity due to the protein in the calculation of the cataphoretic P.D. from the velocity of migration. When the concentrations of the protein exceeded 1/16 per cent, the influence of the increased viscosity had to be taken into consideration. The results of the experiments are given in Fig. 7. At pH 11.0 and 5.8 the addition of albumin did not alter the cataphoretic P.D. of the collodion particles at all. To judge from the experiments with acid dyes, the sodium albuminate could only have acted in this case like NaCl or Na_2SO_4, causing an initial rise of the P.D., but on account of the enormously high molecular weight of the albumin molecule—about 34,000 according to Sörensen—even a 1/16 per cent solution of sodium albuminate could have caused no increase in the P.D. at pH 5.8 or 11.0.

At pH 4.0 and 3.0, where the albumin forms a durable film on the surface of the collodion particles, the negative charge of the collodion particles is always annihilated at a low concentration of the albumin, 1/32,000 and 1/65,000 of 1 per cent respectively, and when the concentration of albumin rises above this value, the particles become

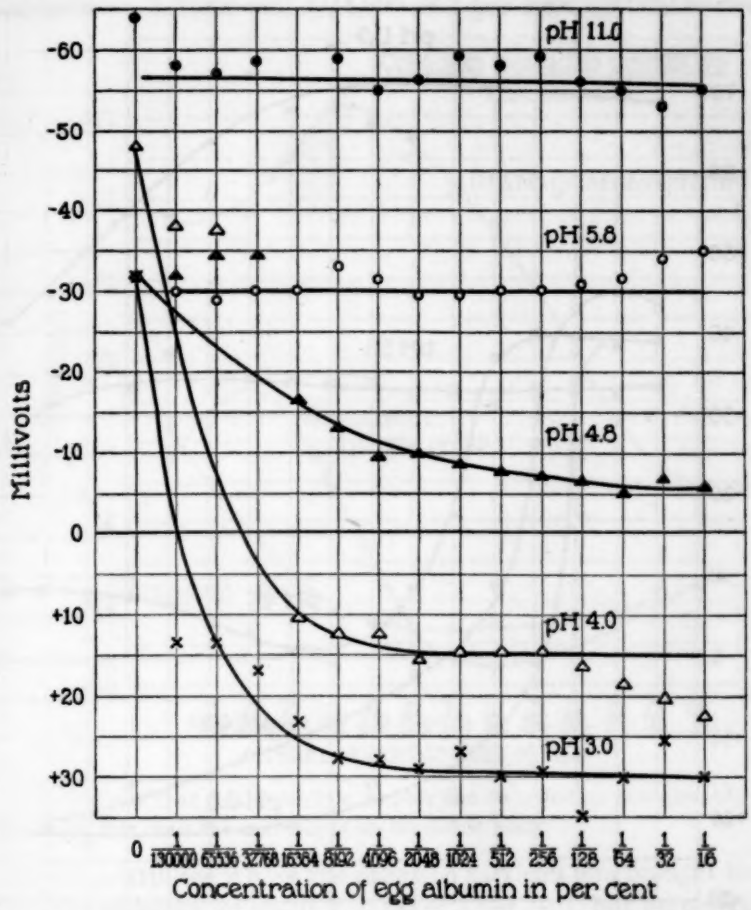

FIG. 7. The cataphoretic P.D. of the negatively charged particles of collodion is depressed by low concentrations of egg albumin of pH 4.8 or below but is not affected by egg albumin at pH 5.8 or 11.0. Low concentrations of egg albumin (1/130,000 and 1/32,000 of 1 per cent respectively) reverse the sign of the P.D. at pH 3.0 and 4.0. At pH 4.8, 4.0, and 3.0 where albumin influences the P.D. it forms a film on the collodion particles.

positively charged. The molecular concentration of albumin where this reversal of the sign of charge of the collodion particle occurs must therefore be in the neighborhood of 10^{-9} M. This is an order of magnitude which possibly approaches that of the efficiency of proteins in immunity and anaphylaxis.

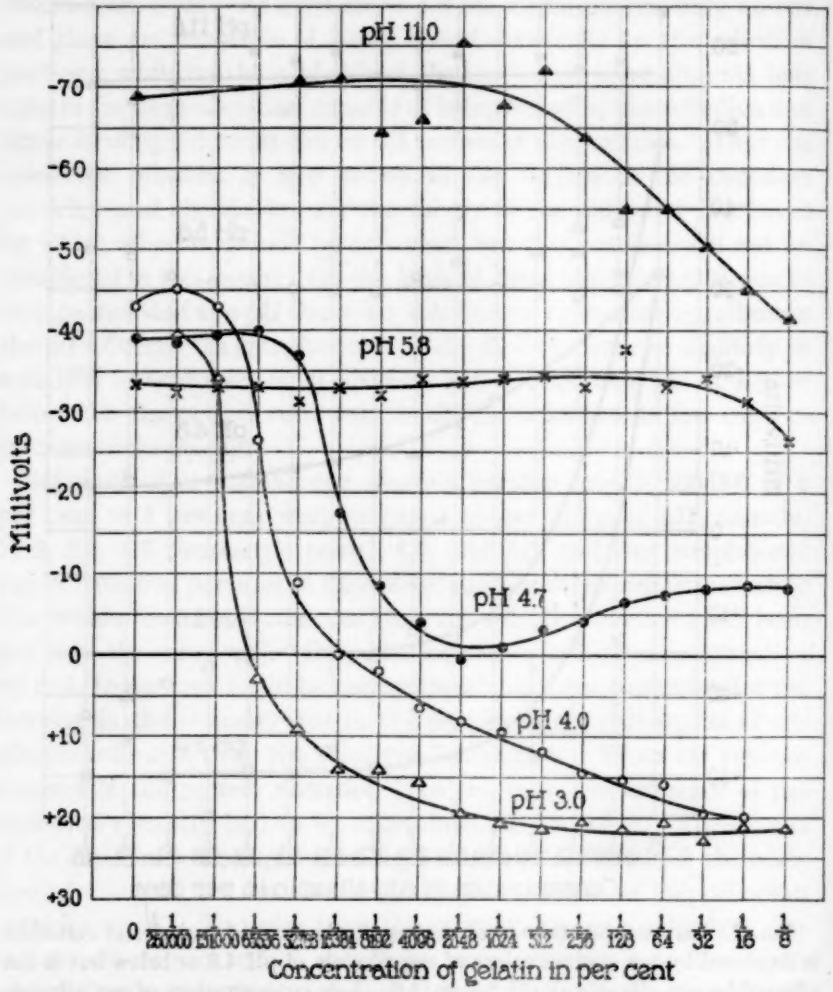

FIG. 8. The influence of low concentrations of gelatin on the cataphoretic P.D. of collodion particles is similar to that of egg albumin in Fig. 7.

Similar experiments were made with solutions of gelatin and it was found that gelatin behaves like albumin, as Fig. 8 shows. The negative charge of collodion particles was reversed in solutions of gelatin chloride of pH 3.0 when the concentration of the gelatin was 1/60,000 of 1 per cent; and at pH 4.0 when the concentration of the gelatin was between 1/8,000 and 1/16,000 of 1 per cent. At the isoelectric

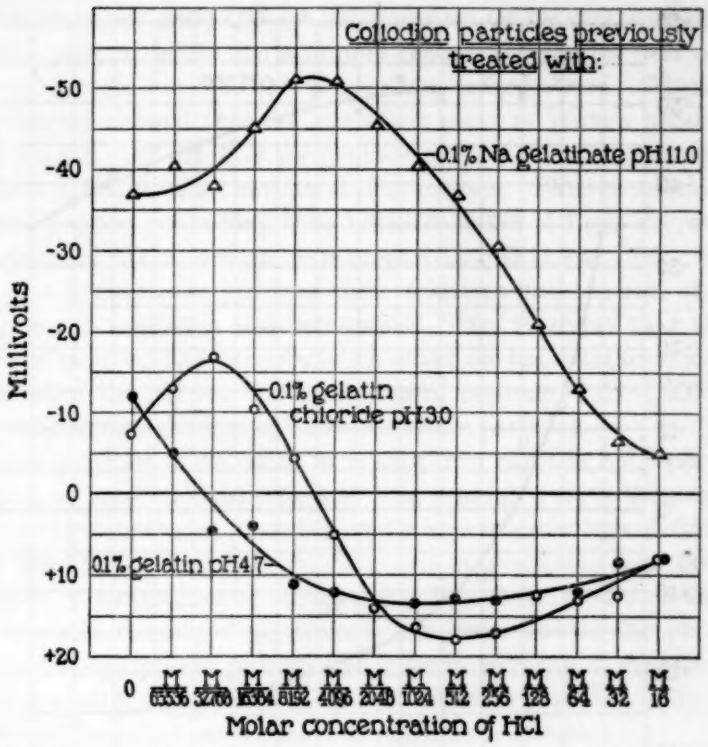

FIG. 9. Proof that gelatin forms a durable film on collodion particles only when it is isoelectric or on the acid side of its isoelectric point.

point of gelatin the P.D. of the collodion particles was brought to zero at a concentration of about 1/2,000 of 1 per cent, but no reversal of the sign of charge occurred.

When collodion particles were left over night in a 1/10 per cent sodium gelatinate solution of pH 11.0 and then separated from the protein solution by the centrifuge, they behaved cataphoretically

like particles not treated with proteins; while when the collodion particles were left over night in a 1/10 per cent solution of isoelectric gelatin (pH 4.7) or of gelatin chloride at pH 3.0, they behaved after being separated from the protein solution with the centrifuge like gelatin particles. This difference could be demonstrated by experiments on the influence of acids on the cataphoretic P.D. of these

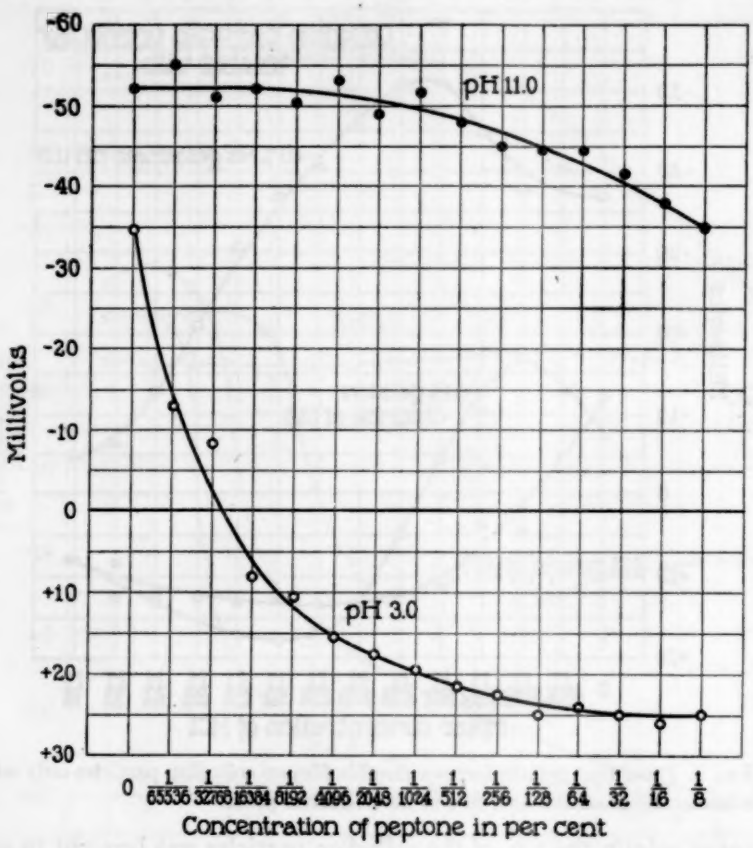

FIG. 10. Peptone acts similarly to albumin. See legend of Fig. 7.

collodion particles (Fig 9). The upper curve in Fig. 9 shows that when the particles had previously been treated with Na gelatinate of pH 11.0 the addition of acid caused no reversal; if there had been a film of gelatin on the collodion particles they would have become

positive under the influence of acid. Such a reversal was observed when the particles had been treated with isoelectric gelatin or with gelatin chloride (the two lower curves in Fig. 9).

V.

It was then intended to find out whether or not hydrolyzed proteins and amino-acids could still act like a protein on the cataphoretic P.D. Witte's peptone acted like a protein though the greater part of the original protein used for this preparation is hydrolyzed. Migration experiments showed that the isoelectric point of Witte's peptone is near pH 4.3. At pH 11.0 the peptone must have existed as anions, while at pH 3.0 it must have existed as cations. Filtered solutions of Witte's peptone containing from 1/65,000 to 1/8 of 1 gm. dry weight of peptone in 100 cc. of water were prepared at pH 11.0 and 3.0. Particles of collodion were put into these solutions and the rate of their cataphoretic migration was determined. Fig. 10 shows that at pH 11.0 the peptone had practically no effect on the cataphoretic P.D., while when the pH was 3.0 a solution of peptone of 1/32,000 of 1 per cent reversed the sign of migration of the particles.

When particles of collodion were kept over night in a 0.5 per cent solution of isoelectric peptone and then separated from the peptone solution by centrifuging, they behaved in aqueous solutions of different salts (NaCl, $CaCl_2$, $LaCl_3$, Na_2SO_4, and $Na_4Fe(CN)_6$) like protein particles, the cataphoretic curves being like those described in the case of casein, denatured egg albumin, etc., thus showing that peptone or the non-hydrolyzed protein left in peptone forms a durable (not easily reversible) film on the surface of collodion particles. This was also corroborated by experiments on anomalous osmosis.

VI.

When, however, experiments were made with solutions of salts of amino-acids like alanine, tyrosine, or leucine, it was found that they always acted like ordinary crystalloidal salts. Since alanine is very soluble, it was possible to work with solutions as high as 1/16 gram molecular. Such solutions of alanine were prepared at three different pH, 3.0, 5.8, and 11.0. Collodion particles were put into the alanine solutions and their rate of migration was measured immediately. At

pH 3.0 the alanine existed as cations; if alanine acted like albumin or gelatin or peptone, it should have reversed the negative charge of the collodion particles.

Fig. 11 shows that no such reversal occurred. Without salt the P.D. of the collodion particles was about 32 millivolts at pH 5.8. Nothing

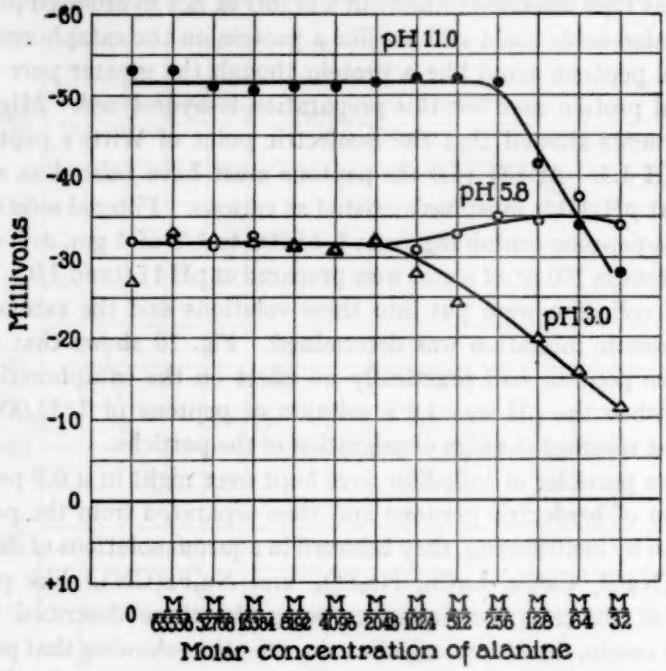

FIG. 11. Alanine acts on the cataphoretic P.D. like ordinary salts of the type NaCl.

changed until the concentration of alanine exceeded M/1,024 when the P.D. dropped. In M/32 the P.D. of the particles was still negative (about 11 millivolts). Hence alanine chloride acted like NaCl but not like a basic dye or protein chloride on the cataphoretic P.D. of the collodion particles.

At pH 5.8 the alanine is practically non-ionized and hence should leave the cataphoretic P.D. unchanged. Fig. 11 shows that this is true within the limits of the accuracy of the experiments. At pH 11.0 the sodium salt of alanine acts as any other salt, *e.g.* NaCl, would at the

same pH on the cataphoretic P.D. of collodion particles, depressing the P.D. when the concentration of sodium alaninate exceeds M/256. There is no rise of the P.D. when the concentration of Na alaninate is

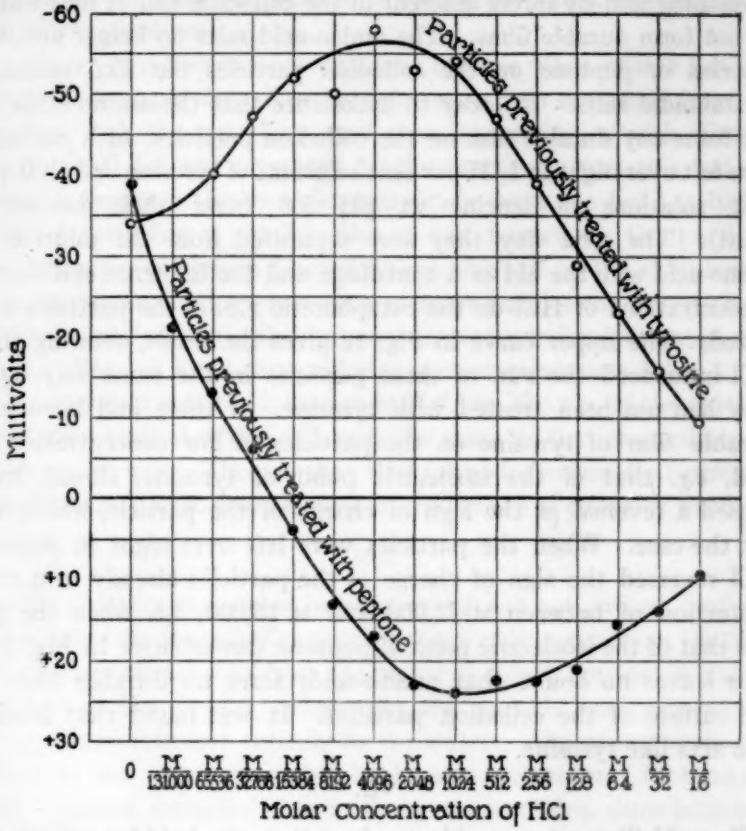

FIG. 12. Proof that amino-acids form no durable film on collodion particles. HCl of different concentrations affects the P.D. of collodion particles kept over night in 1/25 per cent solution of tyrosine in the same way as if the particles had not been treated; while particles treated over night in a solution of isoelectric peptone are rendered positive when the pH of the HCl solution falls below that of the isoelectric point of peptone (4.3).

below this value for the reason that at pH 11.0 the P.D. is already over 50 millivolts if no salt is added, so that salts can raise it only slightly at pH 11.0. NaCl acts in the same way on the cataphoretic P.D. of collodion particles as does Na alaninate.

The experiments with amino-acids seem of crucial importance since they show the difference between the action of ions which form durable films on the collodion particles and hence are attracted into the enveloping film by forces inherent in the collodion and of ions which do not form durable films. The amino-acid salts no longer act like proteins or peptone on the collodion particles but like ordinary crystalloidal salts. In order to make sure that the amino-acids do not form any durable film on the collodion particles, such particles were left over night in 1/25 per cent solutions of tyrosine or 1/100 per cent solutions of leucine at pH 5.8 (near their isoelectric point). The next day they were separated from the solution of amino-acid with the aid of a centrifuge and the influence of different concentrations of HCl on the cataphoretic P.D. of the particles was tested. The upper curve in Fig. 12 gives the result, showing that HCl influenced the P.D. of these particles in the same way as if they had not been treated with tyrosine. If there had been any durable film of tyrosine on the particles, a low concentration of acid, *e.g.* that of the isoelectric point of tyrosine, should have caused a reversal of the sign of charge of the particle, which was not the case. When the particles were left over night in *peptone*, HCl reversed the sign of charge of the particles already in a concentration of between M/32,000 and M/16,000, *i.e.* when the pH was that of the isoelectric point of peptone (lower curve in Fig. 12). This leaves no doubt that amino-acids form no durable film on the surface of the collodion particles. It was found that leucine also acts like tyrosine.

VII.

Since McTaggart was able to show that gas bubbles, which are naturally negatively charged, assume a positive charge in a solution of $LaCl_3$, it is obvious that the forces inherent in the aqueous solution itself can cause an excess of positive ions in the enveloping film. This does, however, not exclude the possibility that La (and Th) ions are also attracted chemically by the collodion particle. The only way of testing this was to find out whether $LaCl_3$ (or $ThCl_4$) acts like basic dyes or like protein chlorides, forming durable films which change the cataphoretic P.D. of collodion particles as do the films of basic fuchsin

or of proteins. If durable films are formed on the collodion particles by $LaCl_3$, it would prove that ordinary salts are dragged into the enveloping film by the chemical forces inherent in the collodion particles; while when no durable film is formed such an attraction is not absolutely disproved.

Particles of collodion were kept over night in 50 cc. of M/8 $LaCl_3$ at pH 3.0 and at pH 5.8. The particles were then separated from the solution with the centrifuge and then washed twice with distilled water of pH 5.8 and separated by the centrifuge from this water in order to separate the particles completely from the $LaCl_3$ solution, without removing a possible film of salt formed on the particles. The influence of various concentrations of NaCl on the cataphoretic velocity of the particles was then measured and compared with the influence of NaCl on collodion particles not previously treated with $LaCl_3$. The pH in all these velocity measurements was 5.8.

It was found that the influence of NaCl on the P.D. of the particles previously treated with $LaCl_3$ was the same as that on particles not treated with salt. There is then thus far no indication that ordinary salts like $LaCl_3$ form durable films on the surface of collodion particles.

VIII.

Solid particles suspended in water which are not ionized (and this is possibly true for collodion particles) can only move in an electrical field if they attract ions from the water or if ions are forced into the enveloping film (which adheres to the particle and moves with it) by forces inherent entirely in the water. The experiments contained in this paper show that both cases may occur. Proteins and basic dyes are attracted chemically by the particles, since both substances form durable films on the surface of the particle. The protein ions as well as the dye cations contribute to the electric charge of the enveloping film adhering to and moving with the collodion particles.

The question arises whether not all the charges of the collodion particles are due to such an attraction of the ions of ordinary electrolytes by the collodion particles. Our experiments show that this is probably not the case, for the following reason. The collodion particles are negatively charged in water and the addition of ordinary electrolytes of the type HCl, H_2SO_4, NaOH, NaCl, Na_2SO_4, $Na_4Fe(CN)_6$,

and $CaCl_2$ increases the negative charge of the particles as long as the concentration of the electrolyte is low. If this phenomenon were due to a chemical attraction between particle and solute, it would mean that the collodion particle attracts anions but not cations. Such an assumption is, however, refuted by the experiments with dyes and proteins which show that only dye cations and protein cations are attracted but not protein anions. In all probability the forces of attraction in this case are not ionic at all since isoelectric protein also forms durable films with collodion particles. There remains then only the other possibility that the negative charge of collodion particles in water and the increase of this negative charge through the addition of low concentrations of electrolytes is due to forces inherent in the water, by which the extreme surface film of water has an excess of anions.

If this reasoning is correct, it leads to several interesting conclusions concerning the orientation of molecules and ions in the surface of the water. It is possible that these forces cause a definite orientation of the water molecules themselves of such a nature that the oxygen molecules of the water are turned outside, while both hydrogen ions are turned inwards. A small fraction of these molecules of water are dissociated electrolytically, so that as a consequence of the orientation of the water molecules the film of water enveloping the collodion particles and moving with them has an excess of anions. When a salt or alkali with monovalent cation is added to the water, the molecules of the solute at or near the surface will also be oriented in such a way that their anions are turned towards the free surface of the water (or towards the collodion particle), while the cations are turned inwards.

This tendency of the cation to be driven farther away from the interface and deeper into the water diminishes apparently with its increasing valency. Thus in the case of salts like $LaCl_3$ or $ThCl_4$ we must assume that it has disappeared completely or that it is even reversed. McTaggart has shown that low concentrations of $LaCl_3$ and still lower concentrations of $Th(NO_3)_4$ suffice to render gas bubbles positive in cataphoresis. This means that the ions of such salts no longer orient themselves in such a way as to turn their anions to the free surface of the water. Their orientation has ceased or may be even reversed. In this case only the forces inherent in the water itself are responsible for the molecular orientation.

In the case of electrolytes with monovalent cation the tendency of the anion to go in the enveloping film of the water may increase with the valency of the anion, though this is not certain.

When an electrolyte is added which forms a durable film on the surface of the particle, the situation is entirely different. In this case the dye cation or the protein cation of the film furnishes part of the charge of the enveloping film, while the other part is furnished by the excess of anions driven into the film by the forces inherent in the water itself.

We do not know at present the thickness of the enveloping film of water which adheres to and moves with the suspended particle; *i.e.*, whether this film is only one molecule deep or whether it is several molecules in thickness. Since ordinary electrolytes like NaCl, Na_2SO_4, etc., raise the surface tension of the water it is possible that their ions do not reach the outermost layer of the water. The question then arises how the molecules of a salt can influence the cataphoretic P.D. We must, in this case, also assume that the molecules of a salt are oriented in the surface layer but that the orientation occurs in a layer beneath the outermost surface stratum. Such an assumption was made by Lenard on the basis of his waterfall experiments, which led him to the conclusion that the outermost surface consists of H_2O molecules and that if salts are dissolved in the water, the cations are nearer to the surface than the anions. Since the addition of little salt increases the negative charge of collodion particles in cataphoresis, it would follow that as long as the concentration of a salt is small and the cations are only monovalent, the anions are nearer the interface than the cations; while with higher concentrations of the salts or with higher valency of the cation this difference disappears or is reversed.

The measurements of the rate of migration of the particles were carried out by the writer's assistant, Mr. M. Kunitz.

SUMMARY AND CONCLUSIONS.

1. The cataphoretic P.D. of suspended particles is assumed to be due to an excess in the concentration of one kind of a pair of oppositely charged ions in the film of water enveloping the particles and this

excess is generally ascribed to a preferential adsorption of this kind of ions by the particle. The term adsorption fails, however, to distinguish between the two kinds of forces which can bring about such an unequal distribution of ions between the enveloping film and the opposite film of the electrical double layer, namely, forces inherent in the water itself and forces inherent in the particle (*e.g.* chemical attraction between particle and adsorbed ions).

2. It had been shown in a preceding paper that collodion particles suspended in an aqueous solution of an ordinary electrolyte like NaCl, Na_2SO_4, $Na_4Fe(CN)_6$, $CaCl_2$, HCl, H_2SO_4, or NaOH are always negatively charged, and that the addition of these electrolytes increases the negative charge as long as their concentration is below M/1,000 until a certain maximal P.D. is reached. Hence no matter whether acid, alkali, or a neutral salt is added, the concentration of anions must always be greater in the film enveloping the collodion particles than in the opposite film of the electrical double layer, and the reverse is true for the concentration of cations. This might suggest that the collodion particles, on account of their chemical constitution, attract anions with a greater force than cations, but such an assumption is rendered difficult in view of the following facts.

3. Experiments with dyes show that at pH 5.8 collodion particles are stained by basic dyes (*i.e.* dye cations) but not by acid dyes (*i.e.* dye anions), and that solutions of basic dyes are at pH 5.8 more readily decolorized by particles of collodion than acid dyes. It is also shown in this paper that crystalline egg albumin, gelatin, and Witte's peptone form durable films on collodion only when the protein exists in the form of a cation or when it is isoelectric, but not when it exists in the form of an anion (*i.e.* on the alkaline side of its isoelectric point). Hence if any ions of dyes or proteins are permanently bound at the surface of collodion particles through forces inherent in the collodion they are cations but not anions. The fact that isoelectric proteins form durable films on collodion particles suggests, that the forces responsible for this combination are not ionic.

4. It is shown that salts of dyes or proteins, the cations of which are capable of forming durable films on the surface of the collodion, influence the cataphoretic P.D. of the collodion particles in a way entirely different from that of any other salts inasmuch as surprisingly

low concentrations of salts, the cation of which is a dye or a protein, render the negatively charged collodion particles positive. Crystalline egg albumin and gelatin have such an effect even in concentrations of 1/130,000 or 1/65,000 of 1 per cent, *i.e.* in a probable molar concentration of about 10^{-9}.

5. Salts in which the dye or protein is an anion have no such effect but act like salts of the type of NaCl or Na_2SO_4 on the cataphoretic P.D. of collodion particles.

6. Amino-acids do not form durable films on the surface of collodion particles at any pH and the salts of amino-acids influence their cataphoretic P.D. in the same way as NaCl but not in the same way as proteins or dyes, regardless of whether the amino-acid ion is a cation or an anion.

7. Ordinary salts like $LaCl_3$ also fail to form a durable film on the surface of collodion particles.

8. Until evidence to the contrary is furnished, these facts seem to suggest that the increase of the negative charge of the collodion particles caused by the addition of low concentrations of ordinary electrolytes is chiefly if not entirely due to forces inherent in the aqueous solution but to a less extent, if at all, due to an attraction of the anions of the electrolyte by forces inherent in the collodion particles.